NOx Analyzer (ENO-30)

User's Guide

Distributed by Amuza Inc: 10060 Carroll Canyon Rd, Ste 100, San Diego, CA 92131,

USA Phone: (888) 680-7775 FAX: (858) 560-8040 Email: info@eicomusa.com

Distributed by Eicom Europe: 8 Terenure Place, Terenure, Dublin, D,WY006, Ireland

Phone: +353 1 902 2700 FAX: +353 1 443 0784

Manufacturered by Eicom Corporation: 113 Kita Enmenden-cho, Shimotoba, Fushimi-ku Kyoto, Japan,

612-8497 Phone: +81-75-622-2112 FAX : +81-75-622-2114

Quick start up and shutdown

Start up

1. Turn on the power switches for the Detector/Oven and Pump Unit. (located on the left side) The oven will start to heat to 35 °C. The pump display with light and you will hear the degasser start, but the pumps won't start yet.
2. Turn on autosampler power. Read panel on left side. You will hear the tray move back and forth as it initializes. The green light at the front will illuminate.
3. Run autosampler initial wash cycle.
4. Use the key pad to set the Carrier pump to 0.33 mL/min and start the Carrier pump first. You may want to start it at a higher rate (0.5 mL/min) to fill the lines and wash out the lines, but reset to 0.33 mL/min for analysis.
5. Now set the Reactor pump to 0.1 mL/min and start the pump. Again you can use a higher setting initially if you prefer.
6. Confirm flow rates are correct by collecting fluid at the waste line. Run each pump separately. Remember the columns should not be installed yet. Collect waste into small microcentrifuge tube for one minute. If it is half the expected volume, then the pump has a bubble and needs to be primed again.
7. Confirm that the Reactor and Carrier solution are routed to the right pumps. If you install the columns when the Reactor and Carrier solution are routed to the wrong pumps, the columns will suffer serious damage. To avoid problems PLEASE BE AWARE OF FOLLOWING:
    - DO NOT put Reactor tube in Carrier bottle.
    - DO NOT put Carrier tube in Reactor bottle.
    - DO NOT run the Reactor pump while the Carrier pump is OFF.
    - When REAC. PUMP is ON, DO NOT disconnect the line from Carrier pumps.
    - When REAC. PUMP is ON, DO NOT open the purge valve.
8. Now you can install the columns. Turn off the pumps. Replace the two way joint with the columns and restart the pumps.
9. Start the Envision software, and monitor the detector signal in the Acquisition Monitor until it stabilizes. (30-60 mins)
10. Inject 10 µL of 10 µM standard solution a calibration chromatogram. The sensitivity of ENO-30 must be check by a standard solution before each analysis. The peak height of NO_2^- should be over *16 mV (10 µL × 10 µM= 100 pmol).*

Short Shutdown, less than 2 weeks

1. Stop the pumps, Reactor first. Wait for 5 min and stop the Carrier pump.
2. Log out of the software.
3. Turn off the power switches on the Detector unit, the Pump unit, and the Autosampler (if installed).
4. Manually flush the pump seal wash with the syringe.
5. Make sure the inlet tubing and waste line are submerged.

Complete Shutdown, over 2 weeks

1. Stop the pumps, Reactor first.
2. Remove the columns (precolumn-separation column-reduction column) as a single piece and stopper both ends.
3. Replace columns with two-way joint.
4. Put Reactor and Carrier inlet tubes in 10% methanol:water solution.
5. Set the pumps to 0.5 mL/min and run them for 20 minutes.
6. Log out of the software.
7. Turn off the power switches on the Detector unit, the Pump unit, and the autosampler (if installed)
8. Manually flush the pump seal wash with the syringe.
9. The inlet and waste tubing should be submerged or capped with the vinyl end caps.

Contents

1. INTRODUCTION ---- 1
1-1. About this user's guide ---- 1
1-2. Important Safety Information ---- 2

2. OVERVIEW ---- 3
2-1. Principle of Measurement ---- 3
2-2. Schematic of Parts and Functions ---- 4
2-3. Tubing Flow Diagram ---- 6
2-4. Pump Seal Wash ---- 7
2-5. Fittings and Tubing ---- 8

3. INSTALLATION ---- 10
3-1. Location ---- 10
3-2. Power Plug ---- 10
3-3. Electrical connections (Detector Unit side panel) ---- 11
3-4 Electrical Connections (Pump Unit Signal Terminal) ---- 12
3-5. Manual injector Tubing Connections ---- 13
3-6. Connect Tubing to Autosampler (optional) ---- 13

4. PUMP UNIT OPERATION ---- 14
4-1. Pump overview ---- 14
4-2. Power On ---- 15
4-3. Key Pad ---- 16
4-4. Display Screen ---- 18
4-5. Log Function ---- 18
4-6. Adjusting the Settings ---- 19
4-7. Priming the Pump/ Purge Valve Operation ---- 22
4-8. Pump error messages ---- 23

5. DETECTOR/OVEN OPERATION ---- 24
5-1. Power Switch ---- 24
5-2. Setting Temperature ---- 24
5-3. Detector Readings ---- 24
5-4. Autozero Function ---- 24

6. ANALYSIS ---- 25
6-1. Accurate Analysis ---- 25
6-2. Water Quality ---- 25
6-3. Reagent Quality ---- 25
6-4. Carrier and Reactor Solutions Preparation ---- 26
6-5. Standard Solution ---- 27
6-6. Waste ---- 27
6-7. Prepare for Analysis ---- 27

6-8. Start up ---------- 28

7. SAMPLE INJECTION ---------- 29

7-1. Manual Injector Operation ---------- 29
7-2. Sample Amount ---------- 30
7-3. Autosampler Operation (optional) ---------- 30

8. SHUTDOWN AFTER ANALYSIS ---------- 31

8-1. Short Shutdown, less than 2 weeks ---------- 31
8-2. Complete Shutdown, over 2 weeks ---------- 31

9. RESTART ---------- 32

9-1 After complete shutdown procedure ---------- 32
9-2. After short shutdown procedure ---------- 32

10. PRECOLUMN ---------- 34

10-1. Determining the Precolumn Condition ---------- 34
10-2. How to Repack the Precolumn ---------- 35
10-3. Filter Exchange ---------- 37

11. SEPARATION COLUMN (NO-PAK) ---------- 38

11-1. NO-PAK ---------- 38
11-2. Identification of Separation Quality ---------- 38
11-3. Washing the Separation Column ---------- 38

12. REDUCTION COLUMN (NO-RED) ---------- 39

12-1. Structure and Maintenance ---------- 39
12-2. Timing to Exchange ---------- 39
12-3. Damage ---------- 39

13. TROUBLESHOOTING ---------- 40

13-1. Pump Problems ---------- 40
13-2. Checking the Flow Rate ---------- 40
13-4. Removing Air Bubbles ---------- 40
13-4. High Carrier Pump pressure ---------- 41
13-5. Unstable Baseline ---------- 41
13-6. Peak Shape ---------- 41

14. MAINTENANCE ---------- 42

14-1. Pump Head Parts ---------- 42
14-2. Changing the Piston Seal and Piston ---------- 43

15. APPENDICES ---------- 47

15-1. Autosampler and Data Processor (EPC-700) Connection ---------- 47
15-2. Specifications ---------- 48
15-3. Sample Preparation ---------- 49

1. Introduction

Thank you for purchasing the Eicom NOx Analyzer (ENO-30). Please read this user's guide before use.

The ENO-30 is an HPLC-based system designed to perform high sensitivity analysis of Nitrite and Nitrate in biological samples. The lower half of the system is the pump unit. It has two independent pumps and a two channel online degasser. The pump has self-learning pulse damping flow control which ensures stable liquid delivery, and is made of inert, non-metallic parts for better salt and acid resistance.

The upper unit uses forced air and a Peltier device to maintain the columns, reaction loop, and detector cell at the correct temperature for the analysis. The detector signal is taken from the side terminals into a data process or (EPC-700) and digitized for analysis in a PC with the Clarity software.

1-1. About this user's guide

- Latest-breaking information may be supplied separately.
- We reserve the right to change the content of this document with or without notice.
- No part of this guide may be reproduced by any means without the prior written permission of Eicom. All rights reserved.
- The ENO-30 requires a thorough understanding of this document for proper operation.
- Please contact Eicom directly if you have any unanswered questions after reading this document.

1-2. Important Safety Information

We insist that users observe the following procedures in order to prevent accidents:

1) Be sure to read and understand this manual completely prior to operating the ENO-30.
2) Follow all warnings listed herein very closely.
3) Do not alter the ENO-30.
4) Never attempt to repair or dismantle the ENO-30 on your own.

⚠ WARNING

This device has been produced for experts who have knowledge of chemical analysis and handling research reagents. Failure to follow instructions may not only lead to poor quality data, but could result in a safety hazard such as fire, electric shock, injury or other damage. DO NOT OPERATE the ENO-30 until reading and understanding this instruction manual completely.

⚠ WARNING

Before you operate the ENO-30 using harmful chemical reagents, be sure to understand its handling methods, physical and chemical characteristics and MSDS (material safety data sheets). Mishandling harmful chemical reagents might result in death or serious injury to the user. To avoid health hazards, wear proper protective gloves, goggles and mask, and be sure there is adequate ventilation. Never allow leakage from any connection points of the tubing.

⚠ WARNING

Flammable chemical reagents must be kept away from sources of ignition and may give off flammable vapors if left uncovered.

2. Overview

2-1. Principle of Measurement

The ENO-30 is a high sensitivity instrument for measuring nitrite and nitrate ion level in biological fluids. This is achieved by combining a colorimetric diazo coupling method (Griess) with the advantages of HPLC.

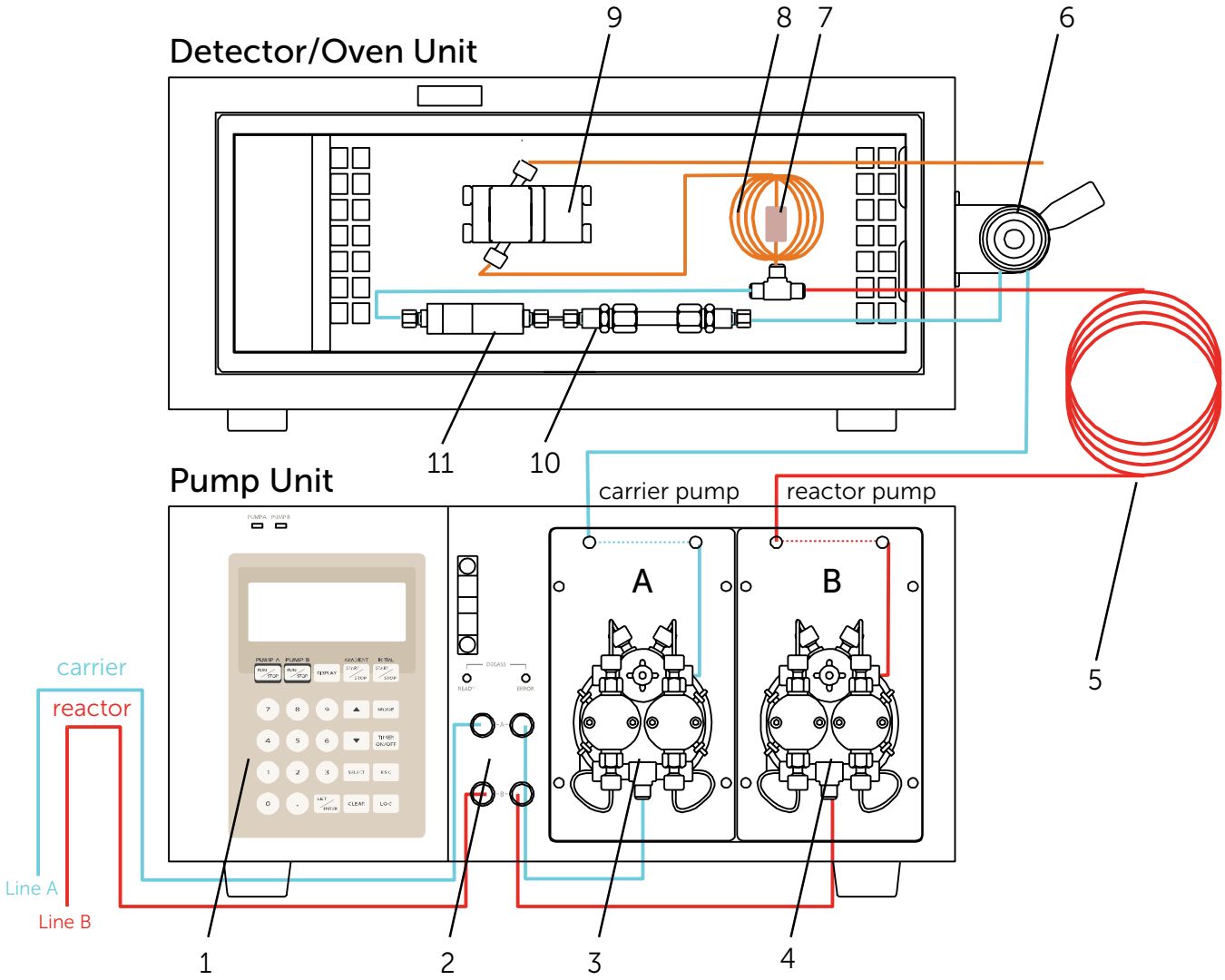
After the sample is injected it is filtered by the guard column. Then the separation column interacts with the ions in such a way that they flow though the column at different rates. Nitrite exits the column first, and then some minutes later, the Nitrate leaves the column. The NO_2^- and NO_3^- next flow into the reduction column made is cadmium and copper.

Inside the reduction column, the NO_3^- is reduced to NO_2^- through a reaction with the cadmium and reduced copper. The NO_2^- that exited first will not react. So now there are two Nitrite peaks, the first is from the Nitrite in the sample and the second is from the Nitrate in the sample. Both are mixed with a Reactor solution supplied by a second pump at a 3-way joint, and then flow into a reaction loop. This gives time for the reaction come to the right temperature and complete formation of a light absorbing diazo compound that can measured at the detector.

In the detector cell, 540 nm (green) light passes through the sample, and the absorption is proportional to the amount of diazo compound, and thus to the amount of Nitrite or Nitrate.

The response of the detector is transformed to a voltage which it generates at the CPU terminal. The time/voltage change is traced by a data processor such as the optional Eicom EPC-700. By comparing peak area or height shown on a chromatogram with a standard one, the exact concentration of Nitrite and Nitrate can be calculated.

2-2. Schematic of Parts and Functions



1. Pump control panel

Set the flow rate of each pump and the upper pressure limit. Then start and stop the pump.

2. Degasser

This degasser removes dissolved air from the Carrier and Reactor solutions before they enter the pumps. It does not work for large bubbles in the inlet tubing. The degasser prevents bubbles from forming in the pumps, which will cause them to stop. The internal volume of each degasser channel is 300 µL.

3. Carrier Pump

It supplies carrier solution to the separation column and reduction column

4. Reactor Pump.
It supplies the reactor solution to the reaction coil.

5. Reactor Backpressure Coil
It maintains high enough pressure that the reactor pump can work efficiently.

6. Manual Injector (and/or Autosampler)
Manual injection of up to 50 µL of sample using a blunt ended Hamilton syringe can be made here.

7. Mixer
Mixes the column effluent with the Reactor solution before entering the reaction coil.

8. Reaction Coil
Gives time for the reaction to be heated and go to completion. It also helps to reduce mixing noise.

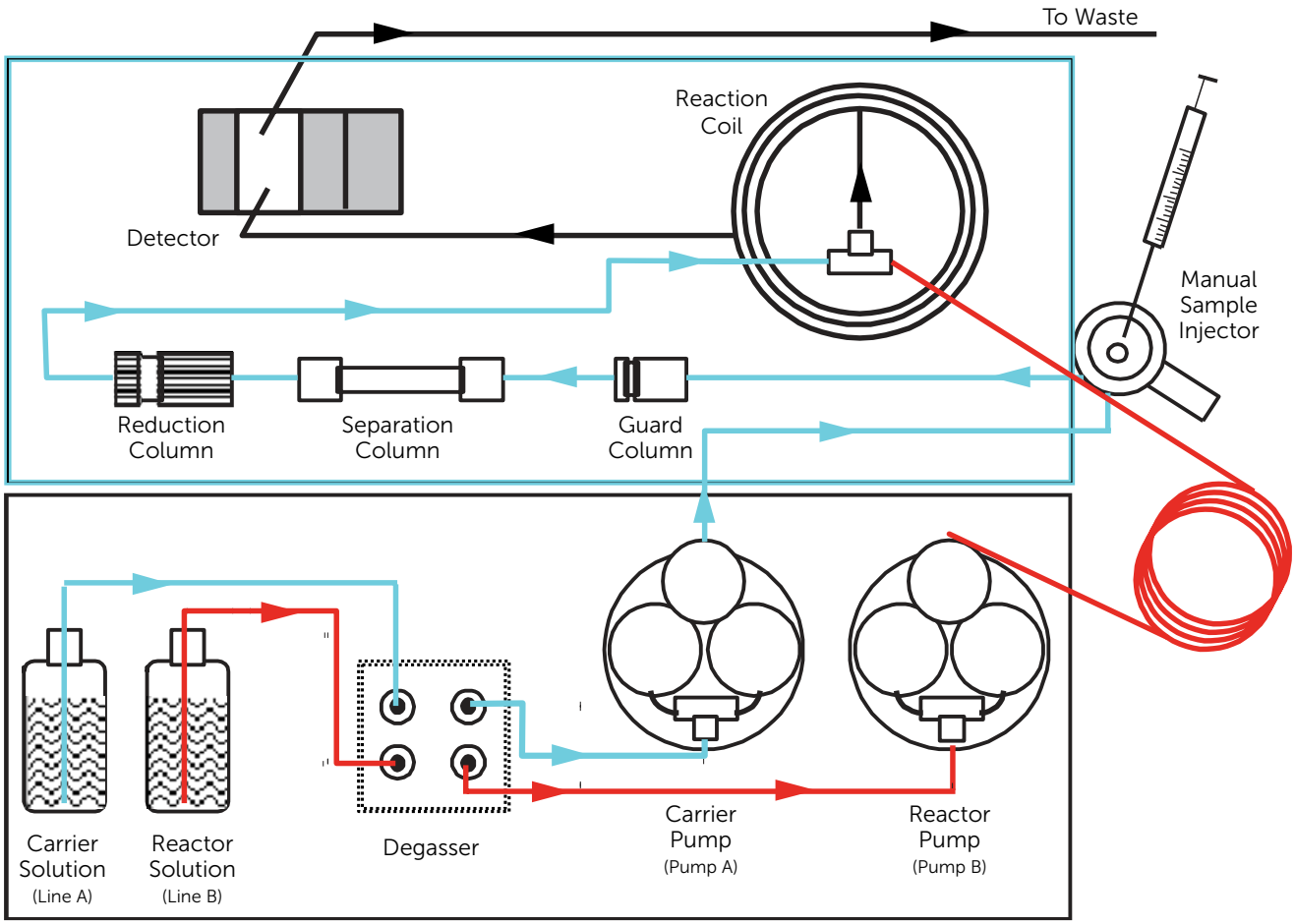
9. Detector Cell
Detects absorbance at 540 nm.

10. Separation Column
Separates Nitrite and Nitrate from other biological compounds.

11. Reduction Column
Reduces Nitrate to Nitrite so that it can react with the Greiss reagent.

2-3. Tubing Flow Diagram

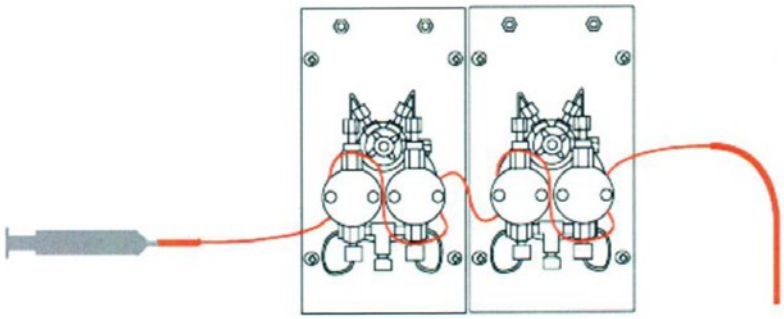
Please use this diagram as a guide in make all the proper tubing connections.



Important points:

- The column effluent (Carrier) and the Reactor solution should enter the 3-way joint on opposite sides of the straight part of the joint to improve mixing.
- Be careful to never let the Reactor solution enter the separation column. It may be severely damaged.
- Also turn the Reactor pump off 1 minute before the Carrier pump in order to wash all the Reactor solution out of the tubing because it can stain if left in place. This will lead to higher background.

2-4. Pump Seal Wash



The ENO-30 has a pump seal wash function, which greatly expands the pump seal lifetime, and prevents damage to the inside of the pump. Small amount of salt containing liquid can leak passed the seal. If it is allowed to crystallize, the salt crystals will scratch the piston and seal, causing premature wear and eventually leakage.

Please purge the washing tube with 2-3 mL of purified water before and after running the pump.

The inlet end of this tube should be set at the bottom of a bottle containing super pure water. The outlet can be routed back to the same bottle or directly to waste. Be sure to change this water frequently to prevent bacterial growth and the buildup of salts and acids.

To begin the flow of water through the wash ports at the back of the pump heads, you must prime the fluid path with the syringe.

1. Aspirate at least 3 ml of water with the syringe.

2. Now you can start the pumps. You should see drops of water flowing from the outlet as the pumps run. If the water doesn't flow easily, the tubing may be clogged or damaged. Check tubing and replace. Leave the syringe in place.

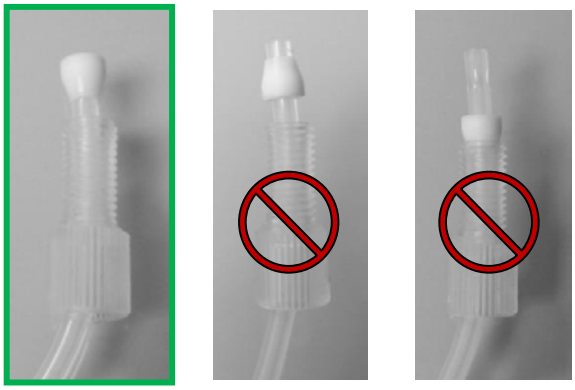
2-5. Fittings and Tubing

There are two main types of fittings used to connect tubing on the ENO-30.

Easy Fit

The Easy Fit connector is for column joints and other high pressure locations. Before tightening the nut, push the tubing end all the way through the Easy Fit connector until it stops inside the connection, and hold it in place while you screw down the nut. The tubing can sometimes be pulled back and not actually be connected. If the tube is not attached properly, the peak shape will be influenced. Be careful not to overtighten because this can cause the tubing to be crushed and the fluid passage blocked.

Flat Seal

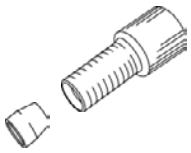
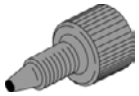
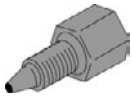


Some connections use a flat seal type of nut and ferrule (white triangle in the figure). Please check the direction of the ferrule, the wide side should be flush with the end of the tubing before inserting into the connection.

Tubing

Material	Dimension	Product Name	Use
PEEK	0.125 mm x 1/16”	PT-12F (red stripe)	Reactor back pressure
PEEK	0.250 mm x 1/16”	PT-25F (blue stripe)	Carrier
PEEK	0.500 mm x 1/16”	PT-50F (orange stripe)	After pumps
PEEK	0.750 mm x 1/16”	PT-75F (green stripe)	Before pumps
PTFE	1 x 3 mm	TFE-1030	Inlet tubing
PTFE	2 x 3 mm	TFE-2030	After degasser
ETFE	0.3 mm x 1/16”	TZ-30	Reaction coil and waste
ETFE	0.8 mm x 1/16”	TZ-80	Seal wash
Silicon	1 x 3 mm	SIL-1030	Seal wash

Tubing and Nut Uses

Tubing Section	Tubing Type	Connector
Inlet Tubing ⇕ Degasser	1 mm x 3 mm PTFE	Connector (Translucent) & Ferrule(White) for 1/8” Flat Seal 
⇕ 3 Way Connector under Pump Head	2 mm x 3 mm PFA	Connector (Black) & Ferrule (Yellow) for 1/8” Flat Seal
⇕ Inlet Check Valve	0.75 mm PEEK (Green stripe)	Fitting 1/16 inch 
Outlet Check Valve ⇕ Purge Valve	0.50 mm PEEK (Orange stripe)	Hexagon-fitting 1/16 inch (Large) 
Pump head Washing Ports ⇕ Seal Washing Tube	0.8 mm x 1/16” ETFE	Hexagon-fitting 1/16 inch (Small)

Caution: *When using the hexagonal fittings*, please fasten by a hand at first and then rotate no more than about 45˚ with a wrench.

Tubing (installation)

Generally, Eicom or its distributor will install the ENO-30 and connect all tubes. In the event that you have to reconnect the tubing yourself, please refer to the following points.

- Connect the outlet tube of the Reactor pumps to the three way joint through a hole in the right side of the Detector unit.
- Connect the outlet tube of the Carrier pumps to the manual injector port “2” (remove the stop fit at the port and connect by Easy fit connector).
- When the Autosampler is installed, the tubing that would normally go to the column inlet should, instead, go to the valve in the autosampler, and then tubing from the Autosampler valve should come back to the column inlet. For further details, refer to the autosampler manual.

3. Installation

The information in this section is given as a reference only. Everything should have been set up by your Eicom Representative during the installation/training. However, if you need to move the instrument or disconnect tubing or electrical connections, this section will be helpful.

3-1. Location

The ENO-30 is designed for use in laboratories for life science.

- ✓ Do not expose to direct sunlight.
- ✓ Install only on a sturdy, horizontal surface.
- ✓ Leave at least 4 inches of space around the instrument.
- ✓ Make sure nothing is liable to fall onto the ENO-30.
- ✓ Do not leave anything on top of the ENO-30.
- ✓ Do not place in a location that is prone to vibration.
- ✓ This product should be operated only in room where there is a minimum of temperature fluctuations. Maintain the temperature between 15-30 °C during use.
- ✓ Do not expose the directly to drafts, including heating and air conditioning vents.
- ✓ Keep away intense heat sources and other equipment that may produce strong magnetic fields or electrical noise.
- ✓ Do not use or store organic solvents or chemicals that emit caustic gases nearby. Always maintain adequate ventilation.
- ✓ The ENO-30 should be operated in a dust-free environment. Remove any dust from around the ENO-30 frequently.

Caution **Do not block the ventilation slits on side of the ENO-30. Excessive heat from the machine may accumulation inside the product and cause damage. Maintain sufficient space around the ENO-30 to ensure adequate ventilation.**

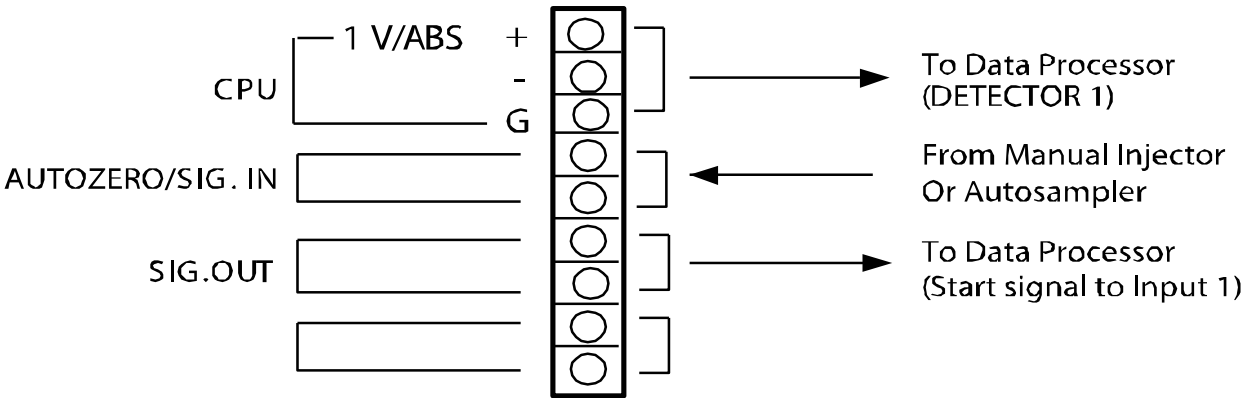
3-2. Power Plug

Power supply conditions of ENO-30 are as follow. Please make sure to use the included triple core cable with grounding wire as power supply.

Power supply Voltage:	AC 100V～240V Single Phase (±10V)
Frequency:	50 or 60 Hz
Power Supply:	2 x 200 W supplies
Connection:	Cable with Grounding Wire (Triple Core)

3-3. Electrical connections (Detector Unit side panel)

The ENO-30 is set up when delivered by Eicom or its distributor. If for some reason, you set up the electrical connectors of the ENO-30 by yourself, please use the diagram below as a guide.



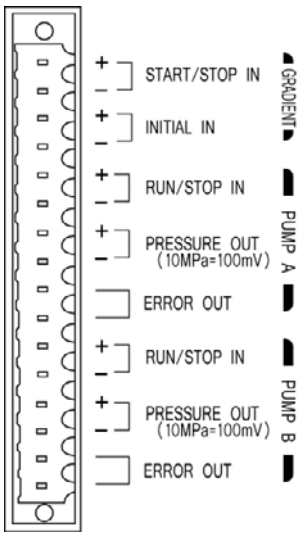
CPU (analog output)
The absorbance is converted to voltage at a ratio of the 1 ABS (absorbance) = 1 V.
This port is usually connected to the Detector 1 signal input of a data processor such as Eicom EPC-700.

AUTO ZERO/ SIG. IN
An external device such as an autosampler or manual sample injector should be connected to this port so that the output signal is reset to zero for each injection. The input trigger from the external device is also sent to the SIG. OUT terminals.

SIG. OUT
This terminal will output a signal when “AUTOZERO/ SIG. IN” is triggered. Connect this position to Input 1 of the data processor. This will start the data collection as each injection is made.

3-4 Electrical Connections (Pump Unit Signal Terminal)

If you have an ***autosampler*** installed this communication signal terminal may be of use to you. Otherwise, you don't need to connect anything here. See below on how the <ERROR OUT> signal can be used.



ERROR OUT — Contact closures will output here if a pump stops because of an error. If you connect to the input channels of the autosampler here and have the input command set to "Freeze", the autosampler will stop making injections when the pump stops. This way you avoid losing precious samples.

RUN/STOP IN — A contact closure signal of more than 10 msec at the <run/stop in> commands will cause the pump to start or stop. This is one way to have the system shutdown automatically after completing analysis.

PRESSURE OUT — If you want to monitor pressure for some reason, this terminal outputs a voltage of up to 100 mV (100 MPa) to communicate the pressure in the system.

3-5. Manual injector Tubing Connections

At the back of the manual injector:

- Port #1 and #4 has the sample loop
- Port #2 receives the tubing from the Carrier pump
- Port #3 goes to the precolumn/separation column, or if installed, to an autosampler. (tubing from the autosampler will come back and connect to the columns)
- Ports #5 and #6 are drain ports (check the crystals don't form on the outlet as that can prevent proper manual injection volumes)

3-6. Connect Tubing to Autosampler (optional)

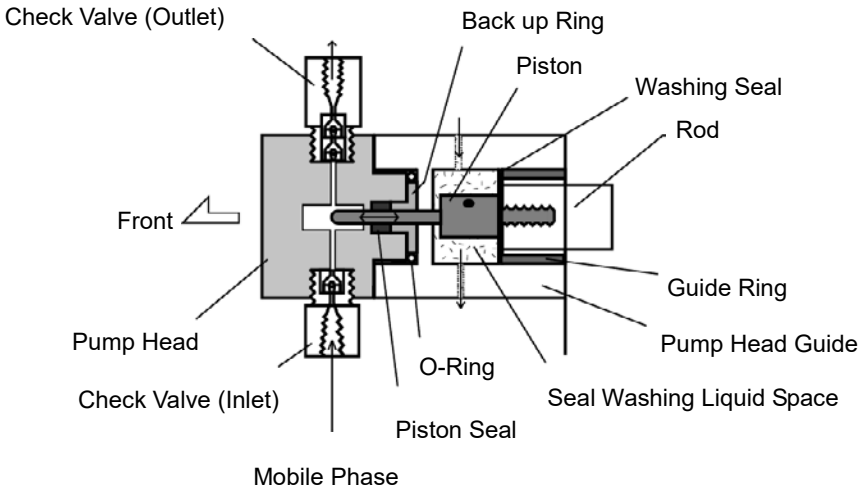
On the valve inside and towards the top of the AS-700 autosampler:

- Port # 2 and #5 has the sample loop
- Port #1 receives the tubing from the port #2 of the manual injector, or if you prefer, directly from the Carrier pump
- Port #6 connects to the columns
- Port #3 connects to the syringe via the buffering tube
- Port #4 is where the sample needle connects

4. Pump Unit Operation

4-1. Pump overview

The ENO-30 has a very precise liquid delivery pump with double pistons. The reciprocal action of the 2 pistons provides for continuous pulseless flow of the mobile phase.



Outlet Check Valve

At the top each side of the pump head (2 per pump), there is an outlet check valve. This valve prevents solution from flowing back into the pump. This is a very sensitive; be very careful to not over-tighten when using a wrench. The internal parts can be crushed and the part will need to be replaced

Inlet Check Valve

At the bottom of each side of the pump head, (2 per pump), there is an inlet check valve. This valve prevents solution from flowing from the pump back to the reservoir. This is very sensitive; be very careful to not over-tighten when using a wrench. The internal parts can be crushed and the part will need to be replaced

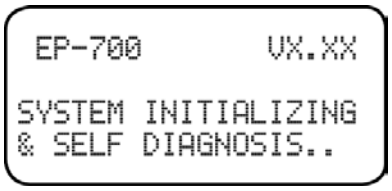
How it Works

As each piston moves forward the outlet check valve opens and the fluid is sent to the columns under high pressure. Then as the piston starts to move back, the outlet check valve closes and the inlet check valve opens so that the piston space can fill with fluid. The cycle is completed as the piston begins forward again; at which time, the inlet check valve closes and the outlet valve opens.

4-2. Power On

Turn on the main power switch on the left side of main body. The display will light up after the power is turned on. A start screen will appear before entering the operation screen. The degasser vacuum pump will immediately start and continue to run on and off throughout operation to maintain a vacuum.

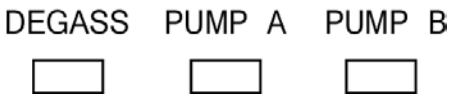
Start Screen



*The EP-700 stores the parameters from the last time the pump was used and loads those upon startup.

Status Indicator

The EP-700 indicator communicates the functional state of the instrument by color, on/off, or blinking.

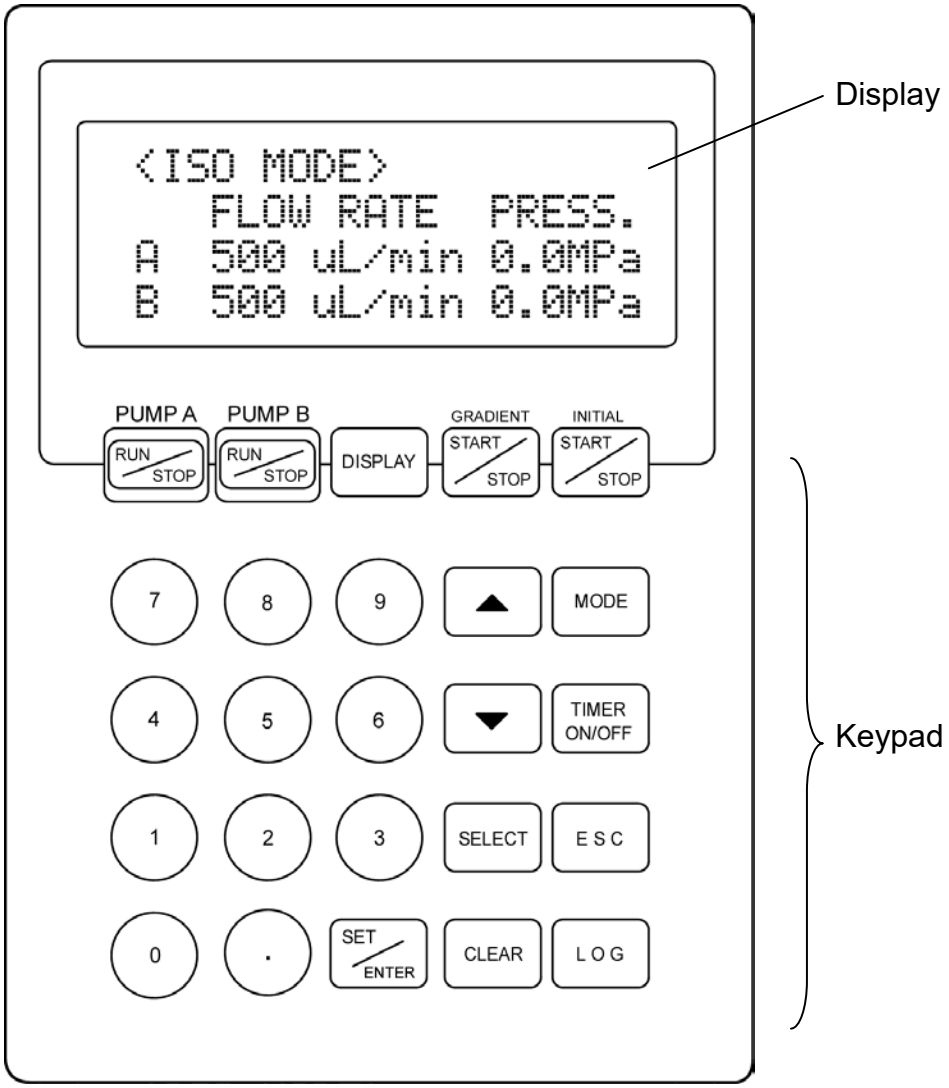


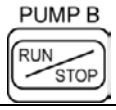
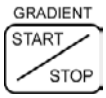
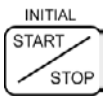
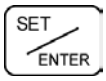
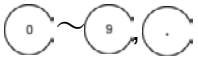
Indicator Lights

Indicator	Subject	Lighting Pattern	
DEGASS	Vacuum pump degasser is running	Turn off	Normal
		Red Blinks	Abnormal *
PUMP A PUMP B	Machine motion of PUMP A and PUMP B	Turn off	Operation Off
		Green Light	Operation On
		Green Blinks	Pump Off timer is in operation

*DEGASS When the indicator blinks red, there is a problem inside the degasser. Please contact Eicom or one of its distributors immediately.

4-3. Key Pad


<ISO MODE>
FLOW RATE PRESS.
A 500 uL/min 0.0MPa
B 500 uL/min 0.0MPa
PUMP A
PUMP B
RUN
STOP
DISPLAY
GRADIENT
START
STOP
INITIAL
START
STOP
7
8
9
MODE
4
5
6
TIMER
ON/OFF
1
2
3
SELECT
E S C
0
.
SET
ENTER
CLEAR
L O G
Display
Keypad

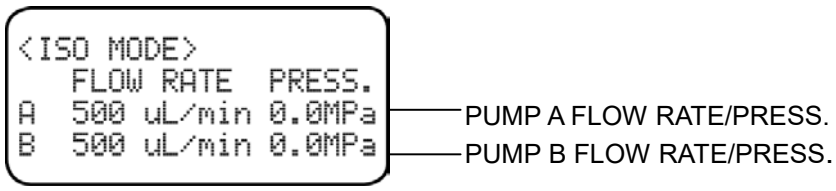
Key	Name	Function
	PUMP A RUN/STOP	In Isocratic Mode, controls the start or stop of pump A
	PUMP B RUN/STOP	In Isocratic Mode, controls the start or stop of pump A
	DISPLAY	Cycles the display between various menus
	GRADIENT START/STOP	In Gradient Mode, controls the start or stop programmed gradient method
	INITIAL START/STOP	In Gradient Mode, controls the start(0min) or stop of a gradient method
	MODE	Switches between Isocratic Mode and Gradient Mode. Only functional when two equal capacity pumps are installed
	TIMER ON/OFF	Start and stop of timer.
	ESC	Cancel parameter input and return to previous menu in the display. Shift from log screen to normal screen.
	LOG	Shift to log screen.
	ARROW	In gradient program enter screen display, controls cursor movement.
	SELECT	Select parameters.
	CLEAR	Correct input parameters while entering them. Delete logs.
	SET/ENTER	Start input/select parameters. Set enter/select parameter
	NUMBERED KEY	Enter numbers.

4-4. Display Screen

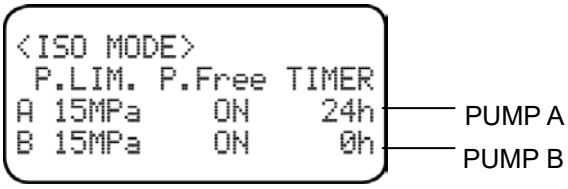
There are 2 screens that can be displayed.

1) “FLOW RATE/ PRESS”

2) “P.LIM/P.FREE/TIMER (Pressure Limit/Pulse Free mode/Timer)”.



<FLOW RATE/ PRESS> Displays the set flow rate and current pressure.

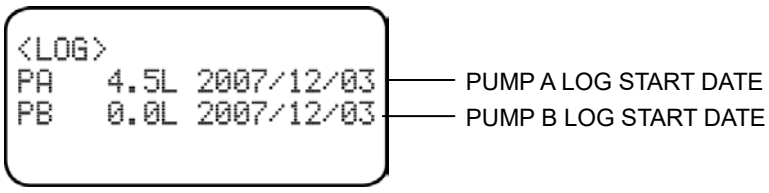


<P.LIM/P.FREE/TIMER> Displays the Pressure Limit /Pulse Free mode*/Timer)

*Pulse free mode is a feature that reduces pressure pulses during pump operation. The pump senses pressure fluctuation, and quickly learns to compensate to reduce pulsing. Please make sure to set this to <PRESET> when doing analysis.

4-5. Log Function

There is also a log function built-in for recording the total liquid pumped from a given start date. It can be used to record maintenance actions, such as installing a new pump seal or column. This log does not have to be set, but we recommend that you use it to aid in troubleshooting and schedule regular maintenance.



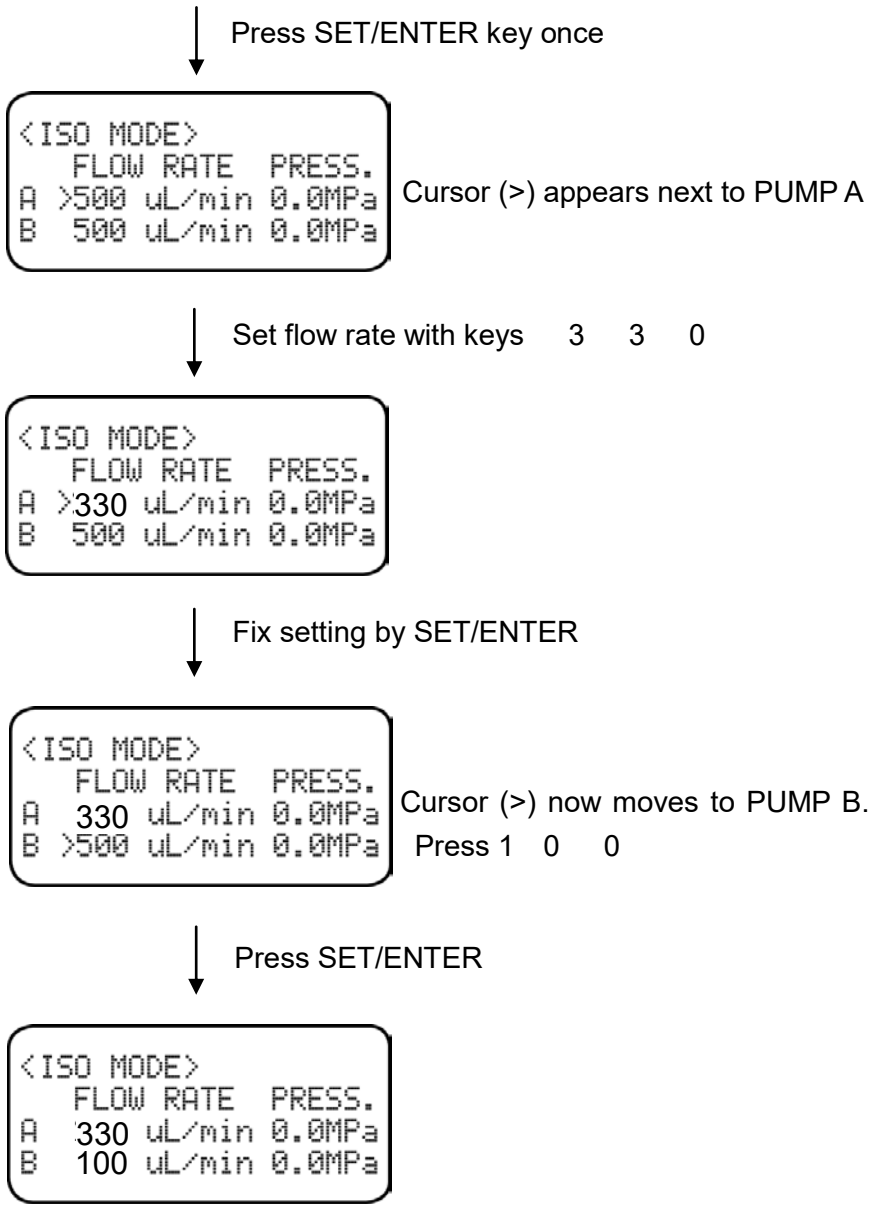
4-6. Adjusting the Settings

Standard pump settings are:

Carrier Pump: 0.33 mL/min	Reactor Pump : 0.10 mL/min
Pressure Limit: 10 MPa	Pressure Limit: 10 MPa

Set the Flow Rate

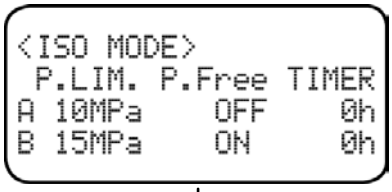
Set the Carrier (PUMP A) flow rate to 0.33 mL/min and Reactor (PUMP B) to 0.10 mL/min.



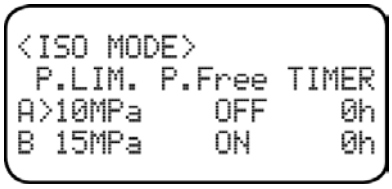
Set the Pressure Limit and Pulse-free Mode

Now set the Pressure Limit for both pumps to 10MPA

Switch screen by pressing DISPLAY

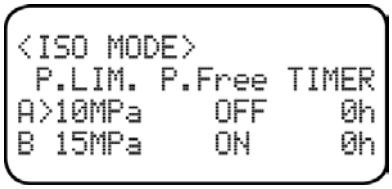


Press SET/ENTER once.

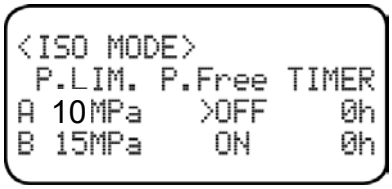


Cursor (>) appears in PUMP A

Enter pressure limit with keys 1 0

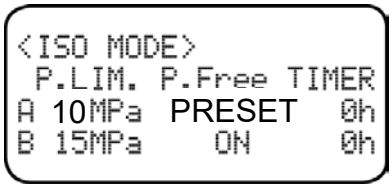


Press SET/ENTER once.



Cursor (>) moves to Pulse Free Mode.

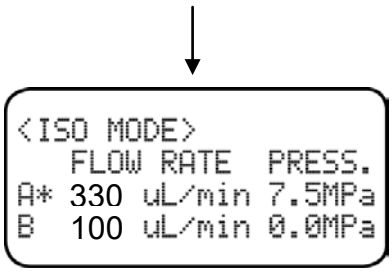
Choose “PRESET” by repeatedly pressing SELECT



Repeat the procedure for Pump B

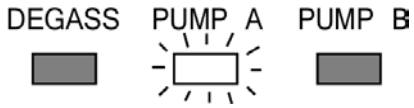
Run the pumps

Press RUN/STOP of PUMP A for starting liquid delivery



"*" appears next to PUMP A to indicate that the pump is running and a pressure is shown

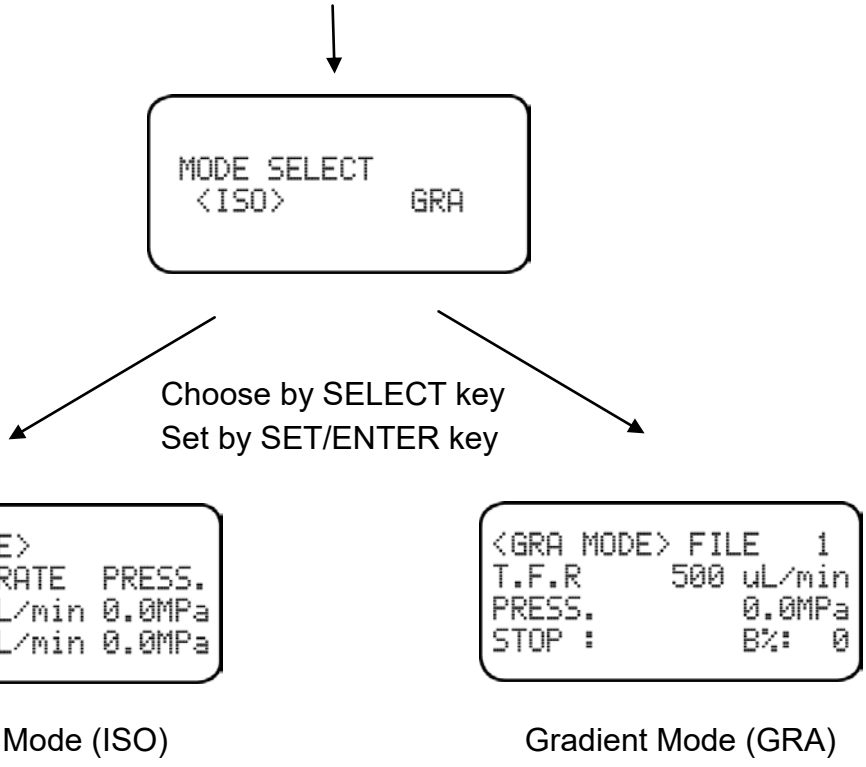
Indicator in main body turns green to show that PUMP A is running.



Use Isocratic mode only

This pump unit also has a gradient function that is not used on the ENO-30. If you accidentally get in to the Gradient Mode <GRA>, you need to switch back to the Isocratic Mode (<ISO>). Use the <MODE> key to switch back.

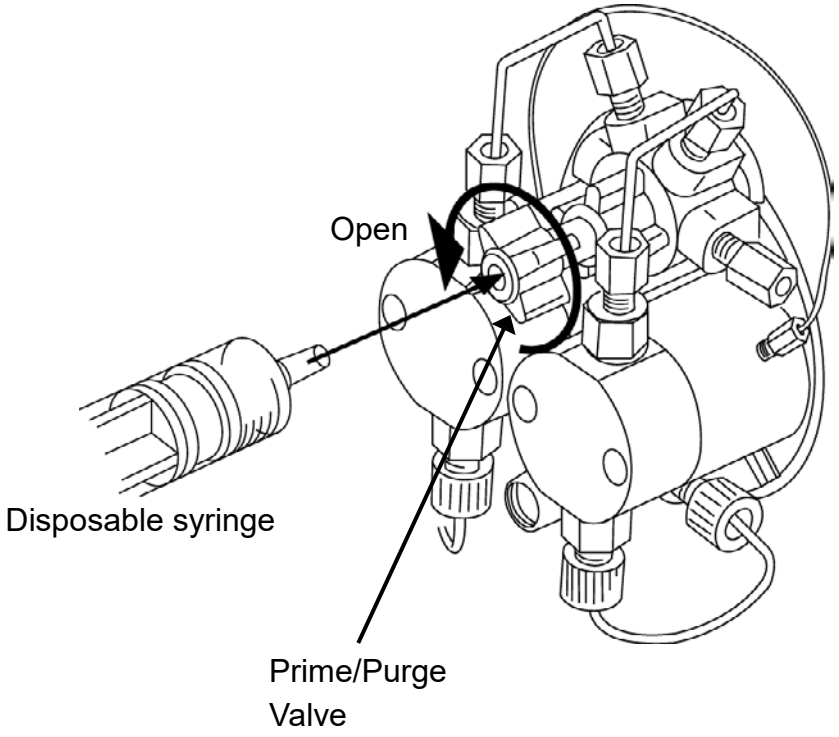
Press MODE key



Isocratic Mode (ISO) Gradient Mode (GRA)

4-7. Priming the Pump/ Purge Valve Operation

The purge valve is used to prime the pump. Air is removed and mobile phase in the degasser and pump is exchanged for new fluid. When the purge valve is open, the pump pushes solution out of the valve, instead of to the column and rest of the system. In order to use the valve, a syringe should first be connected. Then with the syringe still connected, turn the valve just 45-90 degrees counterclockwise to open. Now aspirate to remove fluid from the pump and pull fluid from the reservoir. **(Please note: The tubing downstream from the pump must be connected to a column, precolumn backpressure coil, or be capped for this to work. Otherwise, will be pulled from the open end of the downstream tubing, and not from the reservoirs. You must first block the open end with a cap or a knotted piece of silicon tubing.)**



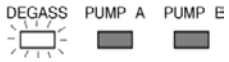
Under normal conditions, internal fluid exchange is complete after aspirating more than 5 mL (Interior content of tubing, degasser, and pump head). The interior volume of the degasser is approximately 300 µL for each channel, not including inner volume of the connected tubing.

4-8. Pump error messages

Error Number	Cause	Handling
ERROR 1 Pressure Limit	Pressure of liquid in pump exceeded pressure limit setting.	Check tubing and columns for a clog, and clear the clog. Check the pressure limit setting. It may be too low.
ERROR 3 Pressure Drop Check Valve	Pressure of liquid in pump decreased rapidly.	Remove air bubble from pump by drawing fluid from the purge valve. The check valve may be stuck. Take it off and shake it. There should be a noise as the ball moves. If not, clean or replace it. Check for leaks in the tubing, and fix any you find.
ERROR 4 Motor Error Torque Overload	Abnormal load detected at the pump drive motor. Abnormal rotation in pump.	Remove air bubble from pump by drawing fluid from the purge valve. The check valve may be stuck. Take it off and shake it. There should be a noise as the ball moves. If not, clean or replace it. Check for leaks in the tubing, and fix any you find.

*Error 3 and Error 4 occur only in Pulse Free Mode ON.

Error message via status indicator light

Indication	Cause	Handling
 DEGASS Blinks Red	Vacuum pressure in degasser is not reaching correct value.	It may require maintenance procedure. Please contact Eicom or its distributors immediately.

5. Detector/Oven Operation

5-1. Power Switch

The power switch is on the left side, not too far back and toward the bottom edge. As soon as the Detector Unit is turned on, the oven will begin to heat. You should feel air blowing inside the chamber. When the door is open, the heat is off, but the fan stays on.

5-2. Setting Temperature

The oven temperature should normally be set to 35 °C. If it is not, you can set it by pressing the set key and then using the arrow key to change the temperature. Press the enter key to confirm. When the temperature of the column oven reaches 35 °C, the green light on the front of the instrument is lit.

5-3. Detector Readings

The display can also show the current readings from the detector, such as Absorbance. Press the Arrow and Enter keys and the same time to display the Absorbance value. Remember, the “absorbance” is calculated by using the difference between the reference light which does not pass through the sample and the light intensity after passing through the sample. To see the individual Reference and Sample light signals press the Arrow and Enter keys again for the Sample value and more time for the Reference value. These values are helpful for confirming if the detector is working properly.

5-4. Autozero Function

You can press the autozero key at any point to manual zero the detector, such as during the stabilization period just after starting the instrument. Remember that the instrument will automatically autozero just after each injection of sample so it’s not necessary to manually operate the autozero.

6. Analysis

6-1. Accurate Analysis

NO_2 exists in the air and dissolves in water. NO_2^- is gradually oxidized to NO_3^- in the solution. Manufacturing processes can also leave plasticware covered in NO_3^- Because most of labs' equipment is polluted with NO_2^- and NO_3^-, we have to pay careful attention to removing contamination, especially for highly sensitive analysis. This pollution is eliminated by a few rinses with non-polluted water (fresh super pure water). The microsyringe and sample injector (manual and external) are easy to contaminate. It is sometimes difficult to make the micro syringe clean. To make it clean, connect a microsyringe to an aspirator and aspirate conc. HCl. Then rise with super pure water. The manual injector, and its injection port, can be cleaned by super pure water flushed by a syringe with a port cleaner attachment. If this flush does not resolve the contamination, disconnect the sample loop and wash the loop with HCl and water. Super pure water is also the candidate of contamination removal. It is better not to store the water; always use fresh super pure water. If storage is necessary, air tight bottle made of glass is suitable for contamination prevention.

6-2. Water Quality

All water used for ENO-30 maintenance and sample preparation must be super pure water (18.2 Mega-Ohm x cm). Distilled or deionized water may be used, although this is not ideal for ENO-30 and accurate analysis. It's best that the water come directly from the water purification system tap. These water quality recommendations help prevent cross contamination of NO_2^- and NO_3^- and prolongs the life of the ENO-30.

6-3. Reagent Quality

- Water; Fresh MilliQ water direct from tap od well maintained system or HPLC grade water. Do not store the water in a plastic bottle. Please use a glass bottle.
- Hydrochloric acid; Analysis or Super grade, 35-37% Hydrochloride.
- Methanol; HPLC Grade
- Standard, reagent grade

6-4. Carrier and Reactor Solutions Preparation

For analysis of NO_2^- and NO_3^-, the Carrier and Reactor should be prepared using Eicom Carrier Powder (NO-CAP3) and Reactor A and B powders (NO-RAP3 and NO-RBP3). Reactor A and B solution are made separately and just enough for one day's analysis is prepare by mixing 1:1.

Carrier Solution

Add 900 mL of water to a 1 liter glass bottle. Add 100 mL of methanol to the bottle. Pour in Carrier powder. Shake to dissolve. Rinse the bottle the powder came in with the prepared Carrier solution to dissolve last of powder and return to prepared solution.

Reactor A

Add 450 mL of water to a 500 mL bottle. Add 50 mL of methanol to the bottle. Add 12.5 mL of concentrated HCL (35-57%). Pour in Reactor A Powder. Shake to dissolve. Rinse the bottle the powder came in with the prepared Reactor A solution to dissolve last of powder and return to prepared solution.

Reactor B

Add 450 mL of water to a 500 mL bottle. Add 50 mL of methanol to the bottle. Pour in Reactor B Powder. Shake to dissolve. Rinse the bottle the powder came in with the prepared Reactor B solution to dissolve last of powder and return to prepared solution.

Complete Reactor Solution

Mix equal parts of Reactor A and B on the day of the analysis.

Storage

Carrier Solution can be stored for several months in the refrigeration. Do not run the system with cold solution because air bubbles can form in the HPLC pumps and cause them to stop.

Complete Reactor solution can only be used for one or two days. Only mix enough for the day. The solution can absorb NO_2 from the air and turn pink. Also cover the bottle with aluminum foil to protect it from light. If the Reactor has turned pink, it cannot be used for high sensitive analysis. If the Reactor is stored in a transparent bottle, it is easier to judge any color change. Do not allow any of the Reactor to enter into columns. Columns will suffer serious damage from contact with the Reactor.

Reactor A and B can be stored for several months in the dark at room temperature or in the refrigerator, but the longer they sit, the low the potency of the complete Reactor solution when it's prepared. It will be immediately pink, leading to lower sensitivity analysis.

6-5. Standard Solution

Dissolve the necessary amount of $NaNO_2$ and $NaNO_3$ in water taken directly from the purified water tap. Then prepare a standard curve by diluting into water, Carrier solution, or the same solution as the sample is in.

6-6. Waste

- Collect Carrier and Reactor Waste into a bottle. This will be mainly an acidic 10% methanol solution with significant cadmium levels.
- The reduction column contains cadmium which slowly resolves into waste. The cadmium in the waste can be concentrated by the method shown below. (If the total mass is small, disposal cost for disposal can be reduced.)

Method to Concentrate Cadmium

1. Add 12 g NaOH for 1 L waste liquid and dissolve.
2. Dissolve 11 g of $FeCl_3$-$6H_2O$ in 100 mL water, add to waste, and mix well.
3. Let precipitate for one day.
4. Filter the precipitate. The remaining supernatant should have low enough cadmium for normal disposal.

6-7. Prepare for Analysis

- Check the amount of Carrier and Reactor. Make fresh Carrier and Reactor as necessary. For high sensitivity analysis, all solutions should be made fresh.
- Aged or pink colored Reactor or very old Carrier will cause high noise generation and reduce sensitivity. If the Reactor solution is stored in a transparent glass bottle covered with aluminum foil, it’s easier to confirm the color of the solution.
- Put the ends of the Carrier and Reactor inlet tubes into the bottom of each bottle. Check degasser and pump markings to be sure. Cover the tops of each bottle with parafilm to prevent dust falling in and to help hold the tubes in place.
- Put the end of the pump seal wash inlet tube into the bottom of a bottle containing fresh super pure water. Prime the seal wash lines before starting pumps.
- Fill the autosampler wash bottle with fresh water or top off the 10% methanol.
- Prepare a fresh precolumn if necessary.
- Make sure the two-way joint is in place of the column.

6-8. Start up

1. Turn on the power switches for the Detector/Oven and Pump Unit. (located on the left side) The oven will start to heat to 35 °C. The pump display with light and you will hear the degasser start, but the pumps won't start yet.
2. Turn on autosampler power. Read panel on left side. You will hear the tray move back and forth as it initializes. The green light at the front will illuminate.
3. Run autosampler initial wash cycle.
4. Use the key pad to set the Carrier pump to 0.33 mL/min and start the Carrier pump first. You may want to start it at a higher rate (0.5 mL/min) to fill the lines and wash out the lines, but reset to 0.33 mL/min for analysis.
5. Now set the Reactor pump to 0.1 mL/min and start the pump. Again you can use a higher setting initially if you prefer.
6. Confirm flow rates are correct by collecting fluid at the waste line. Run each pump separately. Remember the columns should not be installed yet. Collect waste into small microcentrifuge tube for one minute. If it is half the expected volume, then the pump has a bubble and needs to be primed again.
7. Confirm that the Reactor and Carrier solution are routed to the right pumps. If you install the columns when the Reactor and Carrier solution are routed to the wrong pumps, the columns will suffer serious damage. To avoid problems PLEASE BE AWARE OF FOLLOWING:
    - DO NOT put Reactor tube in Carrier bottle.
    - DO NOT put Carrier tube in Reactor bottle.
    - DO NOT run the Reactor pump while the Carrier pump is OFF.
    - When REAC. PUMP is ON, DO NOT disconnect the line from Carrier pumps.
    - When REAC. PUMP is ON, DO NOT open the purge valve.
8. Now you can install the columns. Turn off the pumps. Replace the two way joint with the columns and restart the pumps.
9. Start the Envision software, and monitor the detector signal in the Acquisition Monitor until it stabilizes. (30-60 mins)
10. Inject 10 µL of 10 µM standard solution a calibration chromatogram. The sensitivity of ENO-30 must be check by a standard solution before each analysis. The peak height of NO_2^- should be over *16 mV (10 µL × 10 µM= 100 pmol).*

7. Sample Injection

Always use correct *blunt ended* needle to make injections or risk damaging the valve!

The standard syringe is a 25 µL Hamilton syringe (702SNR). However, a 50 µL syringe may also be used (705SNR).

When the injector knob is moved between LOAD and INJECT, the flow pattern is switched. In the INJECT position the flow from the pump passes through the sample loop and on to the column. The syringe port is connected to one of the drain tubes. The knob should remain in the INJECT position at all times when there is not a syringe inserted in the valve. In the LOAD position, the loop is taken out of the flow and connected to the syringe port. The other end goes to the other drain tube. Do not move the knob to the LOAD position unless there is a syringe in the valve port. When the valve is switched back to the INJECT position, the sample is injected and an electric signal is sent to trigger the autozero function and start data collection. You should hear a beep. Do not connect any other tubing to the drain port in order to avoid to potential troubles.

7-1. Manual Injector Operation

1. Aspirate the sample into a microsyringe. If an air bubble is generated inside of the syringe, please flush out the solution and air bubble and slowly re-aspirate.
2. We recommend that the knob is left at INJECT before and after Injection.
3. Insert a syringe needle until you feel it stop (3 mm further than a weak resistance). *Don't depress plunger.*
4. Slide the knob to LOAD and depress the plunger to load the sample into the sample loop installed on the back of the manual injector. Slow smooth motion is required for best results.
5. With syringe still in place, quickly and smoothly slide the knob from LOAD to INJECT position. By moving the knob from LOAD to INJECT, an electrical signal is also generated and you will hear a beep.
6. After completing these steps, remove the syringe and wash the syringe with super pure water.
7. The needle port of the manual injector must be washed with super pure water using the white port cleaner adapter attached to a syringe.

Caution; *When there is second injection valve, such as an autosampler(AS-700) or online injector (EAS-20s), installed between the manual injector and the column,* the additional injection valve should be set to the LOAD position. This will prevent the sample from going through the sample loop of the second valve as it moves toward the column. If the sample were

to go through the sample loop of the second valve, the sample would spread out and cause a broadened peak.

7-2. Sample Amount

The ENO-30 accepts 1-50 µL of sample or standard. For accurate analysis, the same quantity of sample or standard should be used. It's best if the samples and standard are in the same solutions. Use Carrier solution to dilute samples that are too high.

7-3. Autosampler Operation (optional)

The autosampler is primarily controlled via the Envision software (Eicom EPC-700), more details refer to the Envision Software manual. For complete instructions on the use of the autosampler, refer to the autosampler manual. There are some basic autosampler operations that are covered here.

Before starting any analysis, make sure that the autosampler wash reservoir is filled with 10% methanol. Water may also be used, but in that case, you need to pay extra attention to the reservoir such that it does not have microbial growth. Change the water frequently and use soap to clean.

Run the initial wash cycle to fill the syringe and wash the sample needle before starting any analysis. During this wash cycle, check that there are no bubbles in the syringe. If there are bubbles suck to the syringe plunger, they should be removed before starting the analysis. The fastest way to remove the bubble may be to remove the syringe and let some fluid out past the plunger by pulling the plunger nearly all the way out of the barrel. For more details, refer to the autosampler manual.

During heavy use, it is recommended that the syringe and valve seal should be replaced annually.

8. Shutdown after Analysis

Extended storage period procedures are very important for the ENO-30. If the next analysis will be in less than two weeks, then you can follow the procedure in section 13-1. If it will be more than two weeks, follow the procedure in section 13-2.

8-1. Short Shutdown, less than 2 weeks

1. Stop the pumps, Reactor first. Wait for 5 min and stop the Carrier pump.
2. Log out of the software.
3. Turn off the power switches on the Detector unit, the Pump unit, and the Autosampler (if installed).
4. Manually flush the pump seal wash with the syringe.
5. Make sure the inlet tubing and waste line are submerged.

8-2. Complete Shutdown, over 2 weeks

1. Stop the pumps, Reactor first.
2. Remove the columns (precolumn-separation column-reduction column) as a single piece and stopper both ends.
3. Replace columns with two-way joint.
4. Put Reactor and Carrier inlet tubes in 10% methanol:water solution.
5. Set the pumps to 0.5 mL/min and run them for 20 minutes.
6. Log out of the software.
7. Turn off the power switches on the Detector unit, the Pump unit, and the autosampler (if installed)
8. Manually flush the pump seal wash with the syringe.
9. The inlet and waste tubing should be submerged or capped with the vinyl end caps.

9. Restart

9-1 After complete shutdown procedure

When the ENO-30 was stored as explained at unit 8-2, please prepare for analysis as shown below.

1. Set each inlet tubing in to the Reactor and Carrier bottles.
2. Aspirate from the purge valve on each pump to exchange fluid in the degasser and pump head.
3. Set the Carrier pump 0.5 mL/min and start. Check the flow at the waste outlet.
4. Set the Reactor pump to 0.5 mL/min and start. Check the flow at the waste outlet.
5. If flow is not correct or you receive a pump error message, aspirate at the pump purge valves again.
6. Once pumps are both running, adjust the flow rate of the Reactor pumps to 0.10 mL/min, and then the flow rate of the Carrier to 0.33 mL/min
7. Turn off the Reactor pump and then the Carrier pump.
8. Install the columns and restart the pumps, Carrier pump first.
9. Leave the machine to run for 30 minutes and check the detector baseline by using the Acquisition Monitor in the Envision software.
10. Inject appropriate standard to verify adequate sensitivity for analysis. Typically, 10 µL of 10 µM standard will give peaks of great than 16mV.

9-2. After short shutdown procedure

In case the ENO-30 was left containing Carrier and Reactor for more than just a couple of days or up to two weeks, it may be necessary to clean the system, but first you can check the system condition.

1. Start the Carrier pump and then the Reactor pump.
2. If everything is normal with the pressure, you can let the system run for 30 minutes to stabilize, and check the sensitivity by injecting a standard.
3. *If the pressure is high or the pumps shuts down* because the upper pressure limit is reached, there is probably a blockage somewhere that needs to be cleared. You need to work backward from the waste tubing to determine where the blockage is. (remove the detector inlet, then the reaction loop, then the columns, etc)

4. *If the standard injection doesn't produce good sensitivity* and you have already prepared fresh complete Reactor solution, you will need to clean the system.

Cleaning the system

1. Stop the pumps, remove the columns, and replace with two-way joint.
2. Switch the solution to 10% methanol for both pumps, and run the pumps at 0.5 mL/min for 20 minutes.
3. Now switch the Reactor pump to 100% methanol and Then run the pump for 20 mins at 0.5 mL/min.
4. Switch the Reactor pump back to 10% methanol and aspirate 10 mL or more methanol from the drain valve. Then run the pump for 10 mins, before switching to fresh Reactor solution.
5. Switch the Carrier pump to fresh Carrier solution and run both pumps at 0.5 mL/min for 10 mins.
6. Stop the pumps and set to standard flow rates.
7. Install the columns and start the pumps, Carrier first.
8. Let the ENO-30 stabilize for 30 mins and inject standard solution to check sensitivity.

10. Precolumn

The guard column protects the separation column (P4-NO-PC) from contaminants and prolongs the life of the column. The precolumn traps substances that will stick to the separation column material, and not be able to be removed, before they can damage the separation column. The packing material (NO-PRM) inside the precolumn can easily be replaced. Frequent changes of the precolumn will increase the useable life of the separation column. As a rough estimation, the life span of the precolumn column is about 100 injections of denatured protein samples taken from blood serum plasma or tissue and 200 injections for lower protein samples. These numbers are strongly influenced by sample preparation methods, precolumn packing and so forth. Please refer to unit 15-1. (About sample preparation, please refer to Eicom technical publication entitled “Nitrite and Nitrate Analysis”)

10-1. Determining the Precolumn Condition

If any of the following phenomena occur, it is time to change the precolumn.

- As the precolumn is used, the peak shape loses its symmetry. This means that the back of the peak returns to baseline very slowing and is said to be “tailing”.
- Sometimes if a void develops, or is present after repacking, the peak-tip can be divided.
- The pressure of the Carrier pump will rise as the precolumn’s filter becomes clogged. If the carrier pumps pressure is 1.5 MPa higher than normal, please repack the precolumn and change the upstream filter.

To confirm any suspicion that the precolumn is a problem, please replace the precolumn with a two way joint and inject a standard. If the peak shape improves, the main column is in a good condition and the pre-column should be replaced.

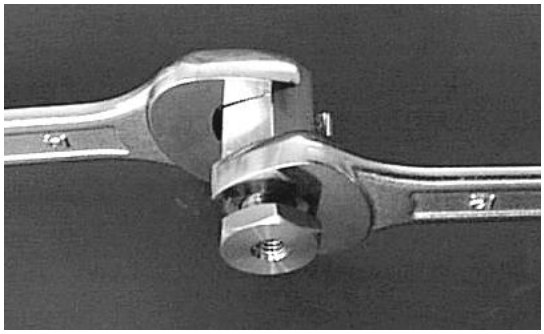
10-2. How to Repack the Precolumn

1) Turn off the reactor pumps and then turn off the carrier pumps.

2) Remove the guard column and replace with a two way joint.

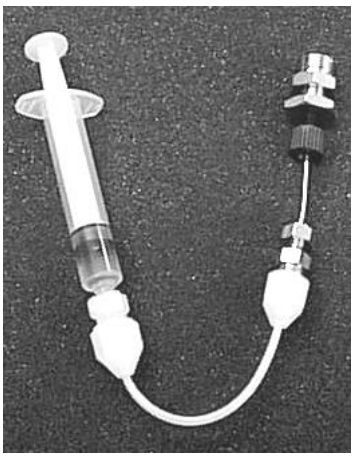
3) Open the precolumn using two wrenches. Then remove the old packing material and wash out the inside of the empty column.

Flow



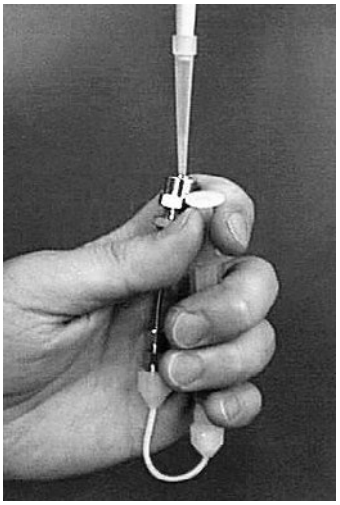
4) Prepare slurry of packing gel by adding about 30 mL of pure methanol to the bottle that the packing gel came in. The exact amount of methanol isn't important. If the bottle has been used before, the methanol may have evaporated. Just add some more, fresh methanol based on the amount of packing material still in the bottle. Cap the bottle and shake. The gel will not dissolve.

5) Connect the 2.5 mL syringe to the lower half of the precolumn via a syringe union and a piece of PEEK tubing.



6) Use a 20-200 µL wide bore pipette (or a normal pipette tip

with the end cut off) to transfer the slurry to the pre-column. Set the pipette volume to 20 µL. Apply the slurry to the pre-column as you slowly aspirate with the syringe. The idea is to pull excess methanol through the column to make room for more slurry, but ***without letting the material go dry***.

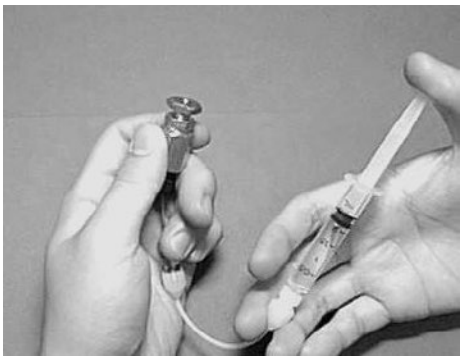


Important

Aspirate with the syringe very slowly and add the next portion of slurry before the methanol goes dry. Always keep a little bit of methanol in the pre-column during the packing procedure. Fill up cavity before inside of a pre-column becomes dry.

7) Repeat these steps until a pre-column is totally filled with the gel. The precolumn has to be filled completely, and so, it's often better to be slightly over filled such that there is a slight mound of gel at the center. Close the pre-column with the two wrenches. The filter can easily fall out of the pre-column while you are closing it. Please take care to prevent this from happening. If you wet the filter before placing to the precolumn, it has a better chance to stay in place.

8) Remove the syringe from the outlet and connect it to the inlet side. Fill the syringe with fresh methanol and flush the column to rinse the new packing material. Then rinse with methanol twice more. Then rinse the pre-column with super pure water in the same fashion. Super pure water will produce a lot of back pressure. Please hold the syringe union onto the syringe.



9) Now you can re-connect the pre-column to the HPLC system. Normally the precolumn will be installed before the column after injectors, except in the case of microdialysis samples where it may be placed before the injector. First connect the tubing to the inlet side only. Pump mobile phase through the precolumn to wash it for several minutes. Then you can reconnect

the outlet side of the precolumn. Failure to pack correctly will cause the peaks shapes to appear strange, except when the pre-column has been place ahead of the injector.

10) If the peak shape of the standard sample doesn't improve with the newly re-packed column, but is still good when only the separation column is in place, the pre-column needs to be repacked again. In most cases, problems with packing are the result of voids in the packing material. Please fill the precolumn slowly. If the correct type of packing material isn't used, the peak shape may appear asymmetric.

10-3. Filter Exchange

When the pressure is high, the upstream filter (PF-04) is clogged and should be exchanged. To remove the filter from the house, you will need to use the filter exchange tool (FP-05, see below). It has a needle that pushes out the old filter as you screw in the tool.

11. Separation Column (NO-PAK)

11-1. NO-PAK

- The size of the separation column (NO-PAK) is ø4.6 mm and 50 mm in length, and it is packed with a styrene polymer gel. The separation quality of column is verified before shipping.
- If methanol should enter into the separation column, the equilibrium with the Carrier will be upset and no peaks will appear. You may have to deliver Carrier to the column for up to 8 hours until retention times are stable. This is sometimes the case with new columns.
- The inside of separation column should not be allowed to dry out. So when storing, the column ends should always be stoppered with stop fit connectors and left at room temperature.
- Generally, the columns pressure against the flow is about 1.0 to 2.0 MPa. Do not expose the column to excessively higher pressures.

11-2. Identification of Separation Quality

As the column is used, the retention time of the peaks will typically decrease and the Nitrite and Nitrate peaks may begin to broaden and overlap. To confirm that the problem is the separation column, remove the precolumn and replace with a two way joint instead of the precolumn. Make a standard injection. If there is no change, then it's probably the separation column that needs to be replaced. In addition, the Nitrite peak, and the negative dip just before it, will not separate well. To check the quality in this case, please use water-diluted standard solution. This makes the negative dip more apparent.

11-3. Washing the Separation Column

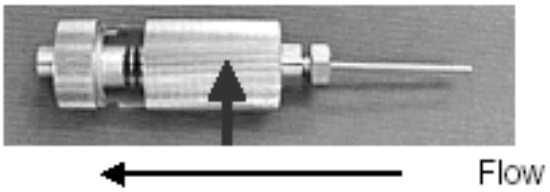
If the quality of the precolumn is maintained as described above, the separation column will not need to be washed. However if the separation begins to show broad peaks because of the separation column, you may want to wash the column to try to improve the separation.

1. To wash, reverse the column direction and do not connect the outlet.
2. Place directly in waste vessel.
3. Now run water at 0.5 mL/min for 1 hour, and then switch to 100% methanol for at least one hour, but you may let it run over night.
4. Then switch back to water for an hour.
5. Now you can put the column back to the forward direction and deliver Carrier for up to 8 hours to re-establish the equilibrium.

12. Reduction Column (NO-RED)

12-1. Structure and Maintenance

NO_3^- is reduced to NO_2^- by the reduction column, but NO_2^- is not reactive with cadmium. The reduction column contains cadmium and reduced copper which are consumed by the delivery of solvent. To fill the space made by consuming of cadmium/copper, tighten the screw to clockwise (arrow in figure) before analysis once a day. If you do not tighten this screw, shape of the peak will become broader.



12-2. Timing to Exchange

The reducing ability is measured by a standard solution. When the NO_3^- peak height becomes extremely low, please replace the reduction column. After attaching a new reduction column, please inject the standard solution. The life span of the reduction column is around 1-3 months.

12-3. Damage

The reduction column loses its ability in following conditions. Please avoid to injecting these samples.

- **AVOID injecting metals with lower ionization tendency than the reducing column's cadmium.** If a high concentration of such a metal is injected, cadmium in the column will be coated by the metal.

  Example: 1mM Hg^{2+}, Fe^{3+}, Nitrosoprusside.

- **AVOID injecting high concentration of thiol compounds.** If a high concentration of a thiol compound is delivered to the reduction column, the compound will be adsorbed on the surface of the cadmium..

  Example: 1 mM Dithiothreitol (DTT)

- **AVOID injecting strong acid.** Strong acid in the samples can prevent the reduction reaction.. However, weaker acids in the sample will be diluted by the carrier, and therefore the will not interfere with the reduction.

  Example: 1 mM Asparatic acid/Ringer's solution (pH4.5), High concentration of HCl, H_2SO_4, H_2PO_4, CH_3COOH

13. Troubleshooting

In the case that your analysis can not be performed by the ENO-30, and that troubles persist after reading this document, please refer to this chapter. *Please inject a standard sample* to find the cause of trouble. If this chapter is not helpful, please contact Eicom.

13-1. Pump Problems

This unit will explain how to determine if the liquid delivery method is normal or not. All delivery problems will appear on the chromatogram chart, usually as a longer retention time than expected. First, check that there are no slow leaks at the tubing connections (around manual injector, valve in autosampler, column inlet/outlet, etc).

13-2. Checking the Flow Rate

For the Carrier pump, set the flow to 500 µL/min and collect liquid at the waste line outlet for 1 minute with **only** the Carrier pump running. If you use a small microcentrifuge tube, you can estimate quickly whether it's 500 µL or more like 250 µL. If it is only 250, then you have a bubble stopping flow through one of the pump heads.

For the Reactor pump, disconnect the tubing where it enters the three way joint by the reaction loop. Collect a sample here for 1 minute by setting the pump to 500 µL/min and running only the Reactor pump. If it's close to 250 µL, there is a bubble.

13-4. Removing Air Bubbles

Air bubbles are the main problem encountered with the pump unit. Air-bubbles are sometimes generated in the Carrier and Reactor Pumps. As long as air exists inside a pump, the pump will not work as liquid delivery pump. To prevent this air generation, an online degasser is included. So air generation will be very rare. However, if air is trapped in one of the pump heads during washing or at start up, the following symptoms may occur.

- lower than expected pressure?
- lower than the expected volume at the waste line outlet based on the flow rate setting?
- longer than expected retention times for the peaks?
- frequent error 4?
- smaller than expected standard peak heights, especially nitrite?

To clear the trapped air bubbles, there are several strategies.

1) The simplest method is to connect a syringe at the purge valve, open the valve and aspirate to remove the bubble. Close the valve and check symptoms again. Repeat a couple of times.

2) If that doesn't work, do the same things again except this time pull the syringe quickly and

release to “pop” the syringe. Then close the valve and check for symptoms again. Repeat a couple of times.

3) The next option is to pull a large bubble through the pump head. Lift the solution inlet tubing out of the liquid and aspirate at the purge valve until there is a large bubble in the tubing. Lower the inlet back in the reservoir and aspirate until the air comes into the syringe.

4) The next method is to force liquid through the pump head by connecting a syringe to the three way joint at the bottom of the pump head. There are details in video available on the Eicom users’ site or directly from your Eicom representative.

13-4. High Carrier Pump pressure

Check the guard column. Remove the guard column and replace it with a two way. Check the pressure. If the pressure is standard pressure, the guard column should be repacked (see chapter 17).

13-5. Unstable Baseline

- Has the system run for more than 30 minutes? It takes time for the system to stabilize and the oven to get to temperature.
- Have you check the pump flow rates? Maybe there is air in one of the pumps.
- Are there any leaks at the tubing connections?

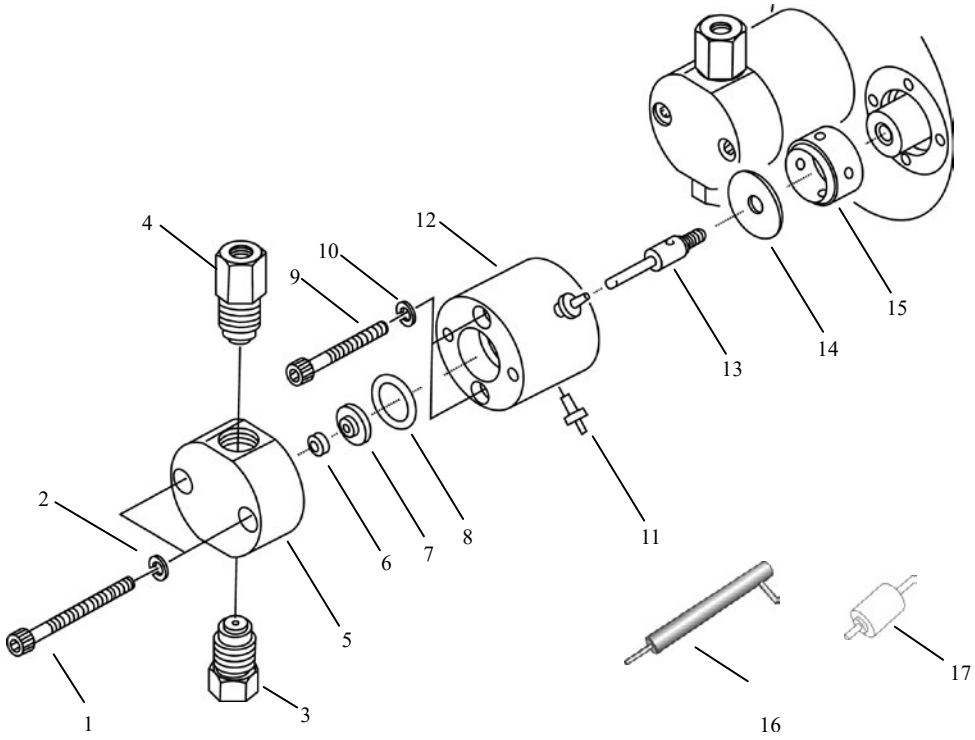
13-6. Peak Shape

Is the peak strange, asymmetric, or tailing? The peak shape is influenced mainly by the separation column and guard column. See chapters 10 and 11.

14. Maintenance

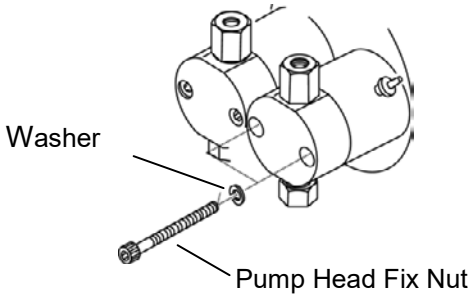
14-1. Pump Head Parts

A parts breakdown of a pump head is as follows.

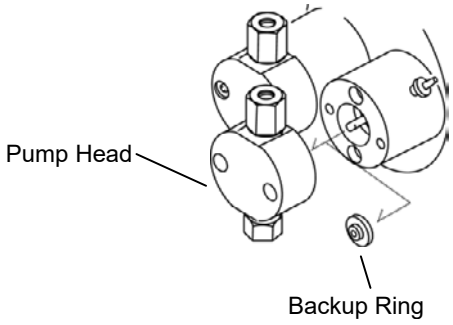


Part Number Part Name
1Pump Head Fix Nut
2Pump Head Washer
3Check Valve (Inlet)
4Check Valve (Outlet)
5Pump Head
6Piston Seal
7Backup Ring
8O-Ring
9Pump Head Guide Nut
10Pump Head Guide Nut Washer
11Washing Port
12Pump Head Guide
13Piston
14Seal for Washing
15Guide Ring
16Piston Replacement Tool
17Piston Seal Replacement Tool

14-2. Changing the Piston Seal and Piston

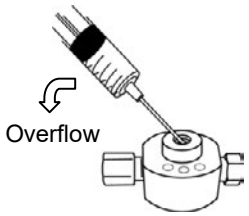


1. Remove tube connectors from the inlet and outlet check valves. Take off pump head by loosening the nuts (2) washer. s you loosen them, alternate between them.

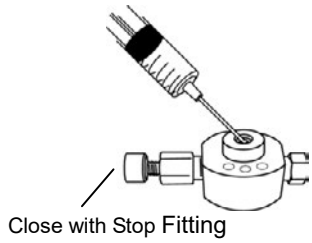


2. Pull out the pump head slowly and in a straight line. The piston is fragile. Be careful to not to break it by twisting or using too much force as you pull out the pump head.

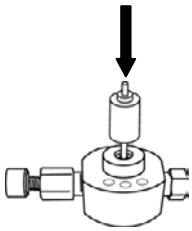
Then, remove Backup Ring.



3. Lay down the pump head; inject water 2-3 mL in the middle of the piston opening. Confirm that waters flows out the outlet check valve.

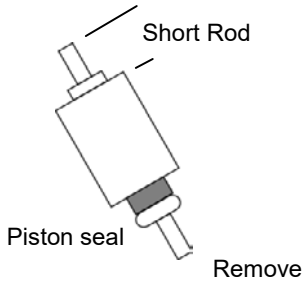


4. Put a stopper fitting in the inlet and outlet valve. Fill the pump head by injecting water until it overflows from piston opening.

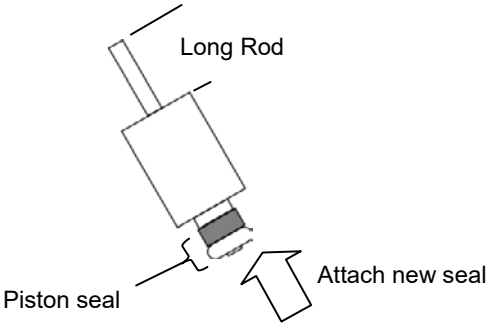


5. Insert the long end of the piston seal replacement tool into the piston opening until the end of the rod reaches the bottom. This should cause the piston seal to be forced back up the rod of the piston seal replacement tool until it is free. Remove the tool and refill the pump head with water.

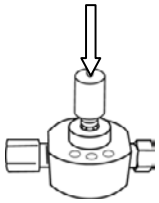
6. Repeat step 5 until the seal is removed.



7. Remove now free old piston seal from the replacement tool.

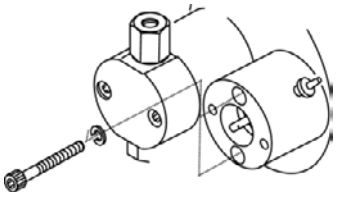


8. To install the new piston seal place it on the short rod of the replacement tool as shown in the figure to the left and wet with water. The plastic part of the seal (not the rubber O-ring) should be pressed against the shoulder of the tool.

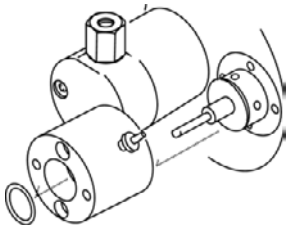


9. Remove stopper fitting from the outlet check valve - the next step will not work if you do not remove the stopper first. Place the new seal over the hole and push it gently into place with the replacement tool. Exchange of piston seal is now complete and you can reinstall the pump head it you don't need to replace the piston.

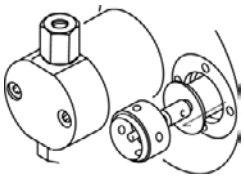
Replacing the Piston



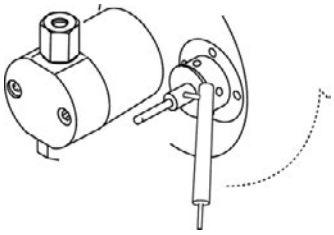
1. With the pump head removed, loosen the screws holding the pump head guide in place. Again alternate between the two screws as you remove them.



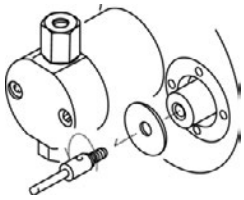
2. Pull pump head guide straight forward. <u>Be careful; the piston is fragile</u>! Sometimes the O-ring comes off at this point. Please be careful not to lose it.



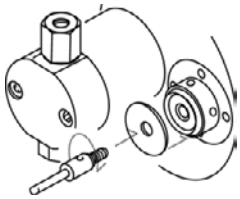
3. Pull the guide ring straight forward to remove it. Do not pull out the washing seal at the same time.



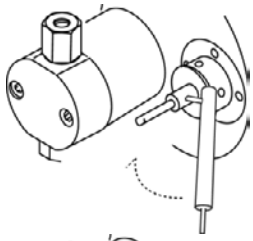
4. Put small rod of the piston replacement tool into hole in metallic part at the base of the piston shaft, and use a counterclockwise motion to loosen it.



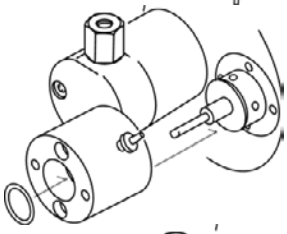
5. After loosen piton, remove it by hand. Then, remove the wash seal as well.



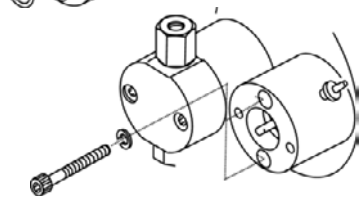
6. Start by attaching the guide ring.



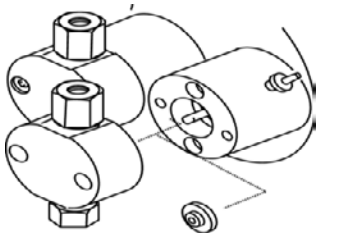
7. Put the washing seal in place and slide the piston through the center hole and screw it in finger-tight.



8. Then use the piston replacement tool to secure the piston.

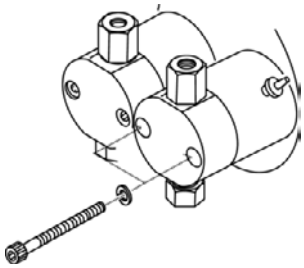


9. Re-attach the pump head guide and O-ring.



10. Tighten up pump head guide nuts once again by alternative between the two screws a little at a time.

11. Attach back up ring and pump head. Pump head should be place onto the piston using a gentle and straight movement. Do not twist. The piston can break very easily. Even, gentle pressure is essential.



12. Tightens up pump head guide nuts by alternative between the two screws until they are completely seated

13. Reconnect the tubing to the check valves, and the process is completed.

15. Appendices

15-1. Autosampler and Data Processor (EPC-700) Connection

1. Disconnect the signal cable of the manual injector (black twisted cable) from the connector located just behind the injector.
2. Connect the AS-700 output (black and red wires from the 3-wire portion of the signal cable) to the AUTOZERO/SIG. IN of ENO-30.
3. Connect the CPU of the ENO-30 to the Detector 1 of the EPC-700 data processor.
4. Connect the SIG.OUT of the ENO-30 to the INPUT 1 of the EPC-700 data processor.
5. Connect the INPUT 2 to the ERROR OUT on the Pump Unit and set the software to use INPUT 1 and 2 to “Freeze”

15-2. Specifications

Detector/ Oven Unit

Detection Principle	Light absorption at 540 nm (diazo compound)
Detection limit	10 nM × 10 µL (0.1 pmol)
Temperature Control	15 °C – 45 °C at room temp of 25 °C
Temperature accuracy	± 0.1 °C
Weight	~ 11 kg
Power	AC100～240V 50/60Hz , 210W
Size	400W x 400D x 145H mm

Pump Unit

Type	Two independent dual reciprocating pumps
Flow Rate	~1 – 750 µL/min
Stroke	4 µL replacement volume
Pulse Damping Function	Actively, computer-controlled, based on pressure feedback mechanism
Maximum Pressure	20 MPa
Pressure Limit	Settable between 0-20Mpa (error 1 triggered)
Piston Material	Sapphire
Other Liquid Contacting Surfaces	PEEK, Sapphire, Ruby, PTFE, PCTFE
Degasser	Two 300 µL loops
Size	400W x 400D x 190H mm
Power	AC100～240V 50/60Hz , 200W
Weight	~ 16 kg

15-3. Sample Preparation

1. Tissue Homogenate

Add methanol at a concentration of 2 mL/g tissue.
Homogenize (in an ice bath, preferentially).
Centrifuge at 10,000 G for 10 min (at 4ºC, preferentially).
Inject the collected supernatant.
Note 1: If the concentration is too high, add the same volume of carrier solution to the supernatant. Note 2. Residual xanthine oxidoreductase in tissue samples may result in NO_2^- levels increasing during storage. This can be prevented by the addition of 0.1 mM oxypurinol to the extraction medium. Please contact Eicom or an Eicom distributor for a detailed protocol for muscle tissue.

2. Blood

Centrifuge at 500 G.
Collect the supernatant (serum or plasma).
Add the same volume of methanol.
Mix using a vortex for 10 sec.
Centrifuge at 10,000 G for 10 min.
Inject the collected supernatant.
Note: Plasma or serum can be directly injected but the precolumn should be changed after every 10 to 20 samples.

3. Cell Culture

The same applies as described in #1 above or by direct injection as below. In the case of direct injection, please change the precolumn frequently.

4. Urine

Dilute with carrier solution to 50 times the volume and then inject.

5. Saliva

Dilute with carrier solution to 10 times the volume and then inject.
Note: The precolumn content should be exchanged after every 50 samples (for samples described in #1-5 above). This is a rough estimation.

6. Microdialysate

Direct Injection: In the case of microdialysate, 100 to 200 samples can be injected per precolumn.
The precolumn content should be exchanged about each 50 sample (for sample #1-5, rough estimate) but microdialysate (6) can be injected 100 to 200 samples for one precolumn.

Limited Warranty

Warranty Period: One year from the original purchase date, as defined by the date of your Eicom invoice. （Except for valve unit-which is warranted for 6 months from the purchase date as defined by the date of your Eicom invoice）.

Warranty Information: During warranty period, this Eicom' product is covered by a limited liability warranty covering manufacture defects. In case that the machine is defective, it will be repaired or replaced free for charge. You will be responsible for return shipping cost. Eicom will cover shipping cost after repairs.

Void of Warranty: The manufacturer's warranty will be void under the following conditions:

1) Failure to follow instructions and warnings relating to product's use.
2) Repaired or altered by anyone other than Eicom.
3) Damage during shipping or transit, or any other accidental damage.
4) Damage due to use of improper voltages.
5) Damage due to improper setup of the equipment.
6) Damage due to any act of God.
7) Any other inappropriate usage of parts or consumables not authorized by Eicom.

All rights reserved by Eicom. Use of images and /or wording of this manual without express permission are completely prohibited. Specifications and descriptions in this user guide are subject to change without prior notice. If you have any further questions, please feel free to contact to Eicom or its distributors