P-FOCUS

IEF Strip System
Electrophoretic
WorkStation-1

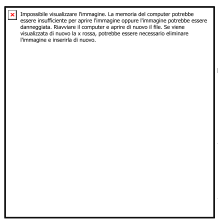
ELETTROFOR
Scientific Instruments

Dimensional®

Instrument User Manual

Index

1. Safety information



WARNING !

The Warning sign highlights an instruction that must be strictly followed in order to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

The Electrophoretic WorkStation (EWS-1) has been tested and complies with the IEC 61010-1 (EN 61010-1) Electrical safety standard.

Extreme caution should be exercised when operate because this instrument can develop higher voltage and current sufficient to produce a lethal shock.

Read this entire manual before using the instrument and use it only according to the manufacturer's instructions.

Before using to make sure that:

- The instrument must always be used with the earth lead of the power cord correctly grounded to earth at the mains outlet.
- To permit sufficient cooling, ensure that the vents in the over and under of the instruments are not covered.
- To use the instrument only indoor, not extreme humidity (above 95%). Avoid condensation by letting the unit equilibrate to ambient temperature when taking the power supply from a colder to a warmer environment.
- Keep the instrument as dry and clean as possible. Wipe regularly with a soft damp cloth.
- Let the power supply dry completely before use. If wetted, unplug the power supply until the instrument is dry.
- Use only undamaged electrical wire and equipment specified for the voltages you will use.
- High voltage electrical wires should be in accordance with IEC 1010-2-031:1993. All equipment connected to high voltage should be in accordance with IEC 1010-1:1993.

2. Function and description

- **Electrophoretic WorkStation–1:**

  The Electrophoretic WorkStation-1 (EWS-1) is an innovative apparatus in the field of electrophoresis (Millioni et al., 2010). It is a versatile modular system which offers a solution for analytical or preparative separations of proteins and peptides by isoelectric focusing (IEF) on immobilized pH gradients (IPG strip), protein SDS-PAGE, DNA electrophoresis. For IEF applications, this instrument can accommodate i) a shorter tray with twelve channels to perform IEF on up to 24 cm long IPG strips; ii) a longer tray with six channels to perform IEF on up to 42 cm long IPG strips (or shorter IPG strips of appropriate pH ranges positioned end-on-end).

The EWS-1 (figure 1) includes the following features:

• two integrated power supplies:

1) High voltage power supply for IEF (up to 15000V and 2mA).
The high voltage power supply is able to deliver a field strength of up to 15000 V, a necessary feature for utilizing IPG strips longer than 25–30 cm. In fact, with such a power supply, it is possible to perform IEF on very long immobilized pH gradients at a voltage gradient of 330 V/cm, a value comparable to present-day power packs operating at 8000 V on 24 cm long strips.

2) Low voltage power supply (up to 250V and 450mA).
A detailed description of the characteristics of these power supplies is reported in paragraph 8.

• temperature control by integrated Peltier elements.

• the integrated control software, with up to ten user-defined IEF protocols, each with up to ten steps per protocol. Programmable functions include: rehydration time,

platform temperature, current limit, voltage limit for each step, voltage gradient or step and step duration.

• a set of strip tray for IPG strips rehydration, running and equilibration. These trays differ in length and number of strip channels.

The EWS-1 must be placed on a flat surface and the safety lid must be properly closed before power is applied.

Figure 1: Electrophoretic WorkStation-1: 1) connectors of the high voltage module (IEF system); 2) connectors of the low voltage module; 3) safety lid; 4) control panel.

➢ **P-Focus**

The only difference between the P-Focus and the EWS-1 is the absence of the low voltage module.

3. Isoelectric focusing applications

Isoelectric focusing (IEF) is a technique for separating molecules by their electric charge differences. It is a type of zone electrophoresis, that takes advantage of the fact that overall charge on a protein is a function of the pH of its surroundings. Proteins carry charged groups on their surface. Each of these functional groups has a pK, which corresponds to the pH at which half of the members of that group are protonated. Above

or below the pK, that group can be considered respectively fully protonated or deprotonated. Thus, as the pH changes, the net charge on a protein's surface will change. The isoelectric point (pI) is the pH at which the net charge of the protein is zero. With the presence of a pH gradient in the IEF method, the protein will migrate to the position where its charge is zero: a protein with a positive net charge will migrate toward the cathode until it meets its pI, while a protein with a negative net charge will migrate toward the anode until it meets its pI. The matrix used in this process has a high porosity to eliminate any "sieving effect", which would cause differing migration rates for proteins of different sizes. If the protein diffuses away from its pI, it will regain its charge and migrate back.

A reproducible pH gradient is crucial for successful IEF. In order to overcome the limitations of carrier-ampholyte-generated pH gradients (e.g. the so-called cathodic drift and plateau phenomenon) immobilized pH gradients (IPG) were developed in 1982. IPGs are based on the bifunctional Immobilines reagents, which are acrylamide derivatives. Their general structure is CH_2=CH-CO-NH-R, where the group R contains either an amino or a carboxyl or group, and forms a series of buffers with different pK values, between 1.0 and 13. Since the reactive end is co-polymerized with the acrylamide matrix, the pH gradients are stable and reproducible also during extended IEF runs. The preparation of IPG strips on a plastic backing offer an optimal solution for convenient handling. The strip length depends on the size of the second-dimension gels to be used, with longer strips and larger gels providing higher sample capacity and resolution.

The main problem to further increase the resolution of IPG strip is the technical difficulty associated with establishing reproducible density gradients over distances longer than 24 cm. This issue could be avoided, by using two or three strips (the number depending on the length of individual strip), with the appropriate pH ranges positioned end-on-end in series (see paragraph 3.2).

3.1 Sample preparation:

Actually, a unique 2-DE protocol of sample preparation that can be applied to all types of samples does not still exists. In fact, although several standard protocols have been published, these methods have to be adapted and further optimized for the type of sample (e.g. microbial mammalian cells) to be analyzed, as well as for the type of proteins of interest (e.g. soluble or insoluble membrane proteins). However, some general recommendations can be given:

- desalt the sample or prepare the sample so that the salt concentration is less than 10 mM. Salt may be removed either by (spin)dialysis or by precipitation of proteins (e.g. by TCA or organic solvents);
- samples containing urea must not be heated (< 37°C) in order to avoid the carbamylation of the proteins by isocyanate formed in the decomposition of urea. Isocyanate covalently modifies lysine residues, thus inducing a change in isoelectric point;
- since IEF in the presence of 8M urea at low temperatures is not suitable due to the formation of urea crystals, optimum focusing temperature is 20°C;
- proteases present within samples have to be inactivated by using protease inhibitors;
- delipidation can be obtained by extraction with organic solvents, such as ethanol or acetone;
- unless present at low concentrations, nucleic acids have to be removed by TCA/acetone protein precipitation or by protease-free RNAses and DNAses digestion;
- the most used sample solubilization buffer are the modified O'Farrell lysis buffer (9 M urea, 2-4% CHAPS, 1% DTT, 2% v/v carrier ampholytes), or the thiourea/urea lysis buffer (2M thiourea, 5-7M urea, 2-4% v/v CHAPS and/or sulfobetaine detergents, 1% DTT, 2% v/v carrier ampholytes);
- the minimum protein concentration should not be less than 0.1 mg/ml, and optimum concentration is 1-5 mg/ml;
- long-time storage of solubilized protein sample is possible in a freezer at -80°C preferred. Repeated freezing and thawing of the sample must be avoided. It is recommended to make aliquots of the sample and thaw only once;

- due to the high dynamic range and diversity of any proteome, it is highly recommended to perform a pre-fractionation step to reduce the complexity of the sample.

Over the past few years, IEF has gained increasingly interest also as a peptide fractionation method (Eriksson H. et al., 2008; Fraterman S et al., 2007; Cargile BJ et al., 2004; Cargile BJ et al., 2005; Geiser L et al., 2011; Heller M et al., 2005). In fact, apart from being an excellent tool for separating complex mixture of peptides, peptide IEF has high reproducibility, is compatible with other fractionation techniques for multistep protocols and can provide the additional information of experimental pI that can aid in the protein and post-translational modification identifications. In this approach, the IPG gel strip is cut into sections and then the focused peptides can be extracted and analyzed from all the gel sections (Cargile BJ et al., 2004). One advantage in working with peptides is that peptides possess greater solubility than proteins. Xiao et al. (2004) demonstrated the efficacy of ampholyte-free peptide autofocusing, avoiding sample losses due to ampholyte removal strategies. The most used solubilization buffer for peptide IEF is 8 M urea, 1% DTT, 5% v/v glycerol.

3.2 Rehydrating and loading IPG strips

- IPG strips must be rehydrated prior to IEF. Usually, protein sample can be applied either by including it in the rehydration solution or by applying it directly to the rehydrated IPG strip via sample cups. We suggest to include the sample in the rehydration solution because, respect to the cup loading approach, this method is technically simpler and eliminates the risk of precipitate formation, which often occurs at the application point of cup loading. The rehydration stock solution can be diluted no more than 1/8 by sample addition. The amount of protein that can be added is dependent upon the length of strip, the pH range and the detection method to be used.
- Select the equilibration tray (figure 2) corresponding to the IPG strip length chosen for the experiment. Wash the extensively with double distilled water to

remove residual protein. The equilibration tray must be completely dry before use.

Figure 2: Equilibration strip tray for 7 cm long IPG strips. Other different trays are available depending on the different length of IPG strips.

- Deliver the solution slowly at a central point in the rehydratation strip tray channel and remove air bubbles.
- Remove the protective cover from the IPG strip (figure 3).

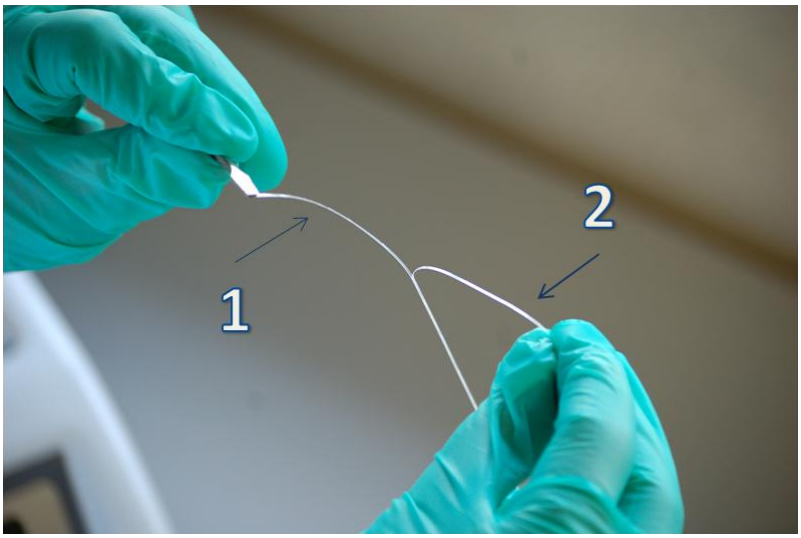


Figure 3: Remove the protective plastic (2) from the IPG strip (1).

- Lay down the IPG strips gel-side down into the rehydration solution. Wet the strip by sliding it back and forth along the tray channel. Remove any large air bubbles. For typical composition of rehydration solution, see paragraph 10.
- Completely cover the IPG strips with the Cover Fluid, and allow the strips to rehydrate. The Cover Fluid is used to minimize evaporation and urea crystallization. A minimum of 10 hours is required for rehydration; overnight is recommended. Alternatively, the rehydration can be programmed as the first step of the IEF protocol. This is convenient if temperature control during rehydration is a concern, or if a low voltage is applied during rehydration.
- Wash the IEF tray extensively with double distilled water to remove residual proteins. The tray must be completely dry before use.

Figure 4: IEF tray for 42 cm long IPG strips or for shorter IPG strips of appropriate pH ranges positioned end-on-end.

- Put the IEF tray on the cooling plate and connect the electrodes to the high voltage module (figure 5, step 1-4).

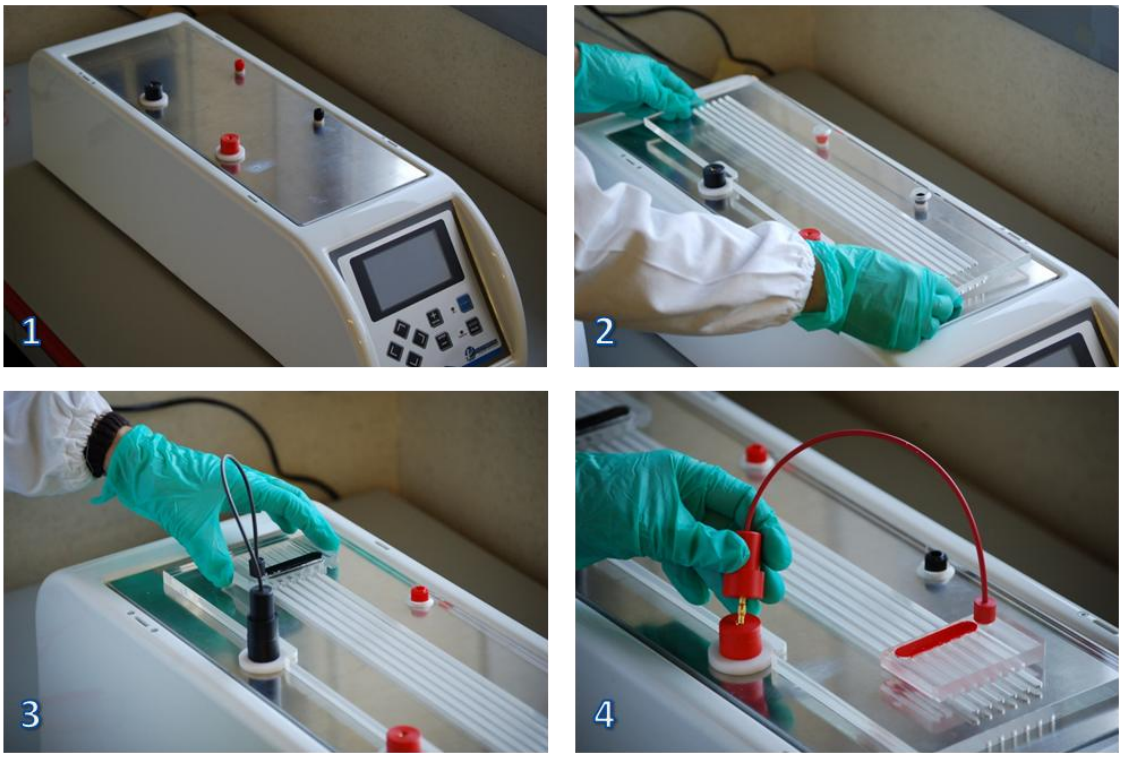


Figure 5

- At the bottom of the teeth of the sliding electrodes, platinum wires provide electrical contact to the small rectangular paper bridges that must be placed at both the anodal and cathodal ends of the rehydrated IPG strips (figure 6). Moistened prior to use with deionized water, these paper bridges absorb excess water, salts, and proteins with pI values that lie outside the pH range of the IPG strip. Transfer the IPG strips from the rehydration to the IEF tray. The IPG strip acrylamide must be in contact with the paper bridges. The acidic end of the IPG gel strips must face towards the anode.

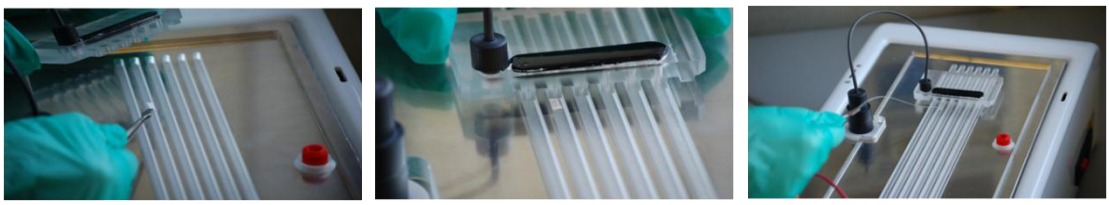


Figure 6: Positioning the paper bridge, the electrode and the IPG strip. These operations must be done at both the ends of the strip. Remove excess water from the paper bridges by blotting with tissue paper: paper must be only wet, not saturated or dripping.

- The sliding electrodes are fully adjustable to suit the length of strips. Since it is very difficult to produce more than 24 cm long pH gradients, shorter IPG strips of appropriate pH ranges can be positioned end-on-end (Poznanovic S et al., 2005). During IEF, proteins efficiently migrate from one IPG to another by traversing buffer-filled porous bridges between the serial IPGs. A variety of materials can function as bridges, including paper or polyacrylamide gels or even the same IPG strips, as shown in figure 7.

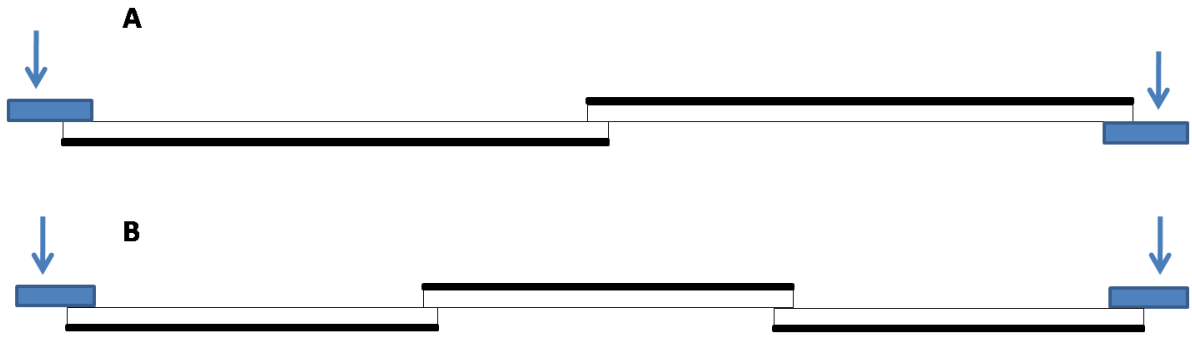


Figure 7: A schematized example to show the loading of two (A) and three (B) IPG strips. The plastic backing film is indicated by a heavy border on the IPG strips. Arrows indicate the position of the electrodes. The small blue rectangles indicate the paper wicks.

- After positioning of both the electrodes, completely cover the IPG strips with the Cover Fluid.
- The instrument must be placed on a flat surface and the safety lid must be properly closed before power is applied. Turning on the mains power switch located on the side of the EWS-1 and of the P-Focus activates a self diagnostic program that runs for approximately 10 seconds.

CAUTION! Always wear protective gloves when working with IPG strip.

Note: After IEF, IPG strip can be stored at -20 °C or lower for several days. For 2-DE applications, SDS-strip equilibration must be performed immediately prior to the second-dimension run, never prior to storage.

4. Low voltage electrophoresis applications (for EWS-1 only)

- The IEF tray can be removed and replaced with small electrophoretic chambers, for other applications besides IEF such as the electrophoresis of DNA on agarose gels (an example is reported in figure 8).
- Connect the electrodes to the low voltage module.

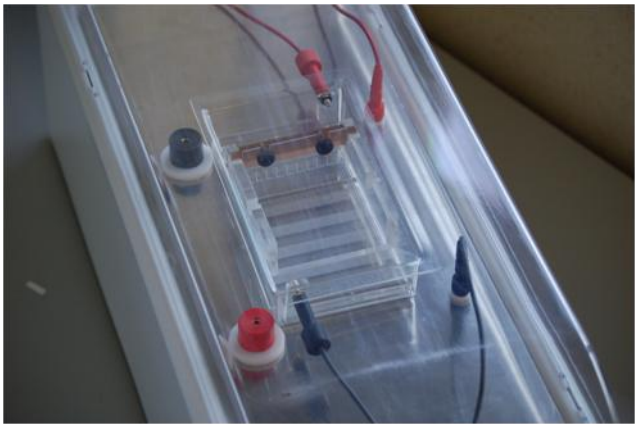


Figure 8

5. IEF Control Software manual

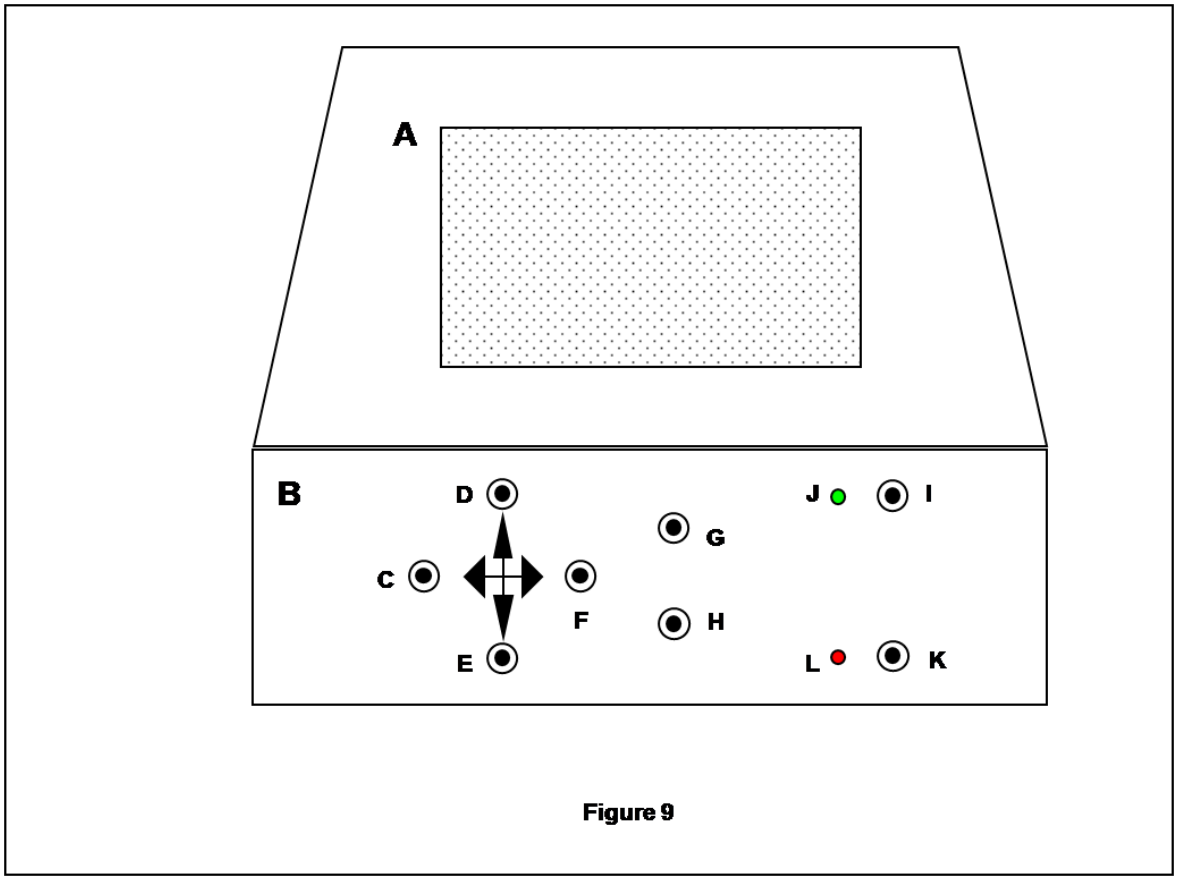


Figure 9

Figure 9: DESCRIPTION

A: LCD Display

B: Control keyboard

C: key **LEFT** (◄)

D: key **UP** (▲)

E: key **DOWN** (▼)

F: key **RIGHT** (►)

G: increase key/selection **+ / ENTER**

H: decrease key/selection **─** / **ENTER**

I: **START key**

J: green LED – if on, machine is working

K: key **STOP / PAUSE**

L: red LED – if , machine is not working

5.1 General informations about cursor keys

- The cursor keys (UP, DOWN,LEFT and RIGHT) are used to navigate through the display. The current cursor position is indicated by highlighting text.
- Increase/selection (+ / ENTER) and decrease/selection (—/ ENTER) key are used to confirm (for example, if you want to exit from an application and the cursor was placed on"EXIT"). Also is used to change parameters. In most cases, to quickly change a parameter, simply hold down the button.
- The START and STOP/PAUSE keys are active only in "OPERATING STAGE", respectively to start or resume execution of a protocol or to stop it temporarily or permanently.

  To ensure the execution of these actions, you must be press button for a longer time that the normal.

5.2 Plate connectors (for EWS-1 only)

The position of high and low voltage connectors is schematically reported in figure 10.

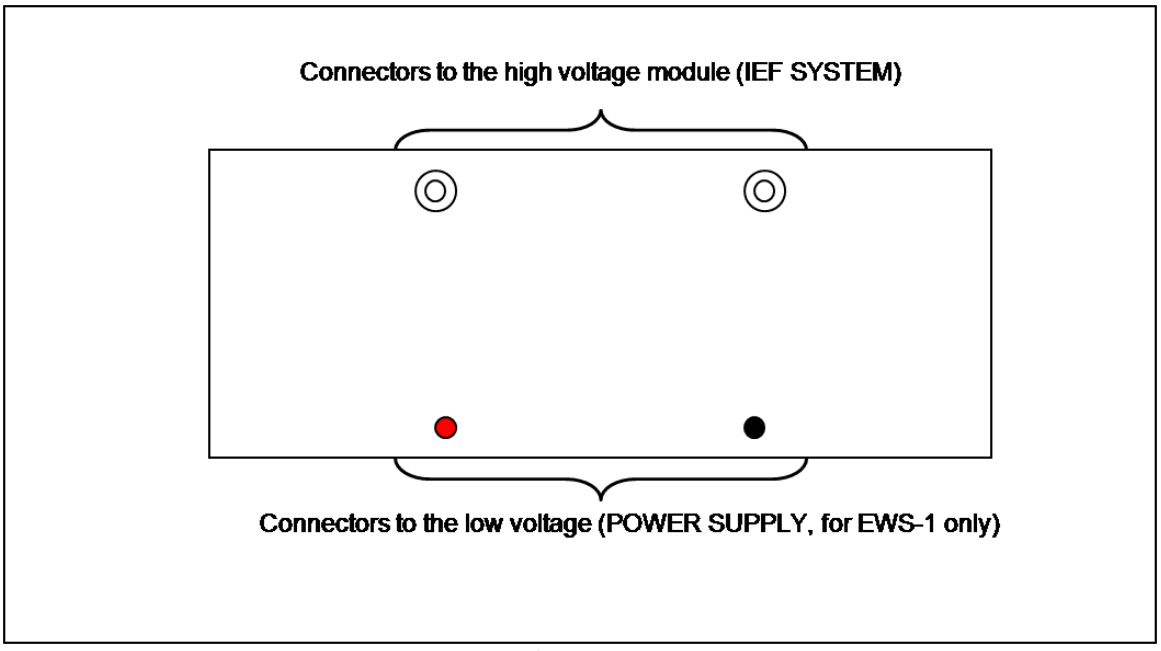


Figure 10

5.3 How to turn on the system

Make sure that the power cord is correctly plugged (100-250, 50-60Hz). Turning on the mains power switch located on the side of the EWS-1 and of the P-Focus activates a self diagnostic program that runs for approximately 10 seconds. If any failure is detected, call Elettrofor service. Press the switch on the right side of the device. After turned on, the system will check and test all the components for a few seconds. The LCD display will show the company logo, both LEDs will be lit (see figure 9: J and L), and an audible signal will be activate. When test operations will be complete, display will show "MAIN MENU ". To turn off the system simply press the switch on the right.

5.4 How to use the system

The EWS-1 Electrophoresis WorkStation has two internal power supplies that can be used in 2 different exclusive mode:

1) High voltage power supply for isoelectric focusing system IEF (up to 15000V and 2mA)

2) Low voltage power supply for normal electrophoresis (up to 250V and 450mA)

5.5 Main Menù

Allowed selections in the MAIN MENU screen:

1.IEF SYSTEM (section relating to the high-voltage)

2.POWER SUPPLY (low-voltage power supply section)

Using UP and DOWN arrow keys, move to the desired menu item. To confirm, press one of the selection key button.

5.6 IEF system

In this section you can access all functions for general setup, protocol setup and the use of the high voltage power supply.

In the menu, "IEF MENU" the following selections are allowed:

- OPERATING STAGE

- PROGRAM SETUP

- SETUP

Use the UP and DOWN arrow keys, move to the desired menu item. To confirm, press one of the selection key button.

5.6.1 Operating Stage

MAIN FEATURES

- In this section you can choose and run a program.
- Before and during the execution of a program you can change the value of some parameters. This changes will be operative only during the current session, because data is not stored on memory.
- The screen shows the name of the program, as it was set in the SETUP PROGRAM section for the specific program selected.
- The REHYDRATION PHASE can be included or excluded from the current program, in which case you can change the duration time.
- Before running selected program you must set the strip number that will be used (STRIP NO.) for the current session.
- Each step include a voltage value (VOLT), a duration time (TIME), an operative mode (MODE) and a remaining time (REMAIN.TIME), which indicate how much time is left to ending the current STEP.
- For each program, the system will calculates the total duration (under theTIME column) and the total remaining time (REMAIN below the column TIME). These are the two TOTAL TIME and are composed with time associated to the STEP and the rehydration phase (if included).

PROGRAM SELECTION

Move the cursor key on the program number (NR PROG.) and use the + and ─ to select the desired number program.

REHYDRATION PARAMETER

- **Before the execution of a program.** Alongside "REHYDRATION", total duration of rehydration phase is displayed. If the values of hours and minutes are different from zero, this means that the program is running with the rehydration time equal to the previous time set up. If the values of hours and minutes are 00:00, the rehydration phase is not included and protocol will begin with the " first non-zero STEP".

- **During the execution of a program**. In this case, alongside ”REHYDRATION”, will be displayed the value of remaining time. If rehydration phase is included and is currently running, you have the possibility to change the remaining time.

If this time is set up at 00:00, it means that you want to end prematurely the rehydration phase, this change is implemented after few seconds. Instead, If the remaining time is increased, this is only possible if the total duration of rehydration phase does not exceed the maximum allowed: Length + Total Elapsed Time = Time Remaining <99:59.

STRIP NUMBER AND POWER LIMITATION

- Before starting a program, you must set the strip number (STRIP N^{O}.) You can enter values between 1 and 12.
- When setting this parameter, a check to the maximum power required is automated made in relation to the maximum current per strip set in the “SETUP” section. It would be possible that the maximum number of strip number set is less than 12.

PARAMETERS VIEWING AND CHANGING

- On entering this section, you will see the table containing STEP 1 to 5.
- To see next 6 / 10 STEP, move the cursor in one step 5 parameters and press DOWN, or move to “EXIT” and press the UP button.
- In addition, during the execution of a protocol, the cursor can be moved only within the range that goes from STEP (or rehydration) up to the first “zero-STEP”.
- Changing the STEP parameters: if protocol is not running, for each step you can change the voltage (VOLT), and if this is different from zero, the values of step duration (TIME) and the operative mode (MODE).
- During the execution of a protocol is possible to change the voltage (VOLT), and if this is different from zero, the remaining time (REMAIN.TIME)

PROTOCOL EXECUTION

- To start a program, press START button for a few seconds, red LED will turn off and green LED will lights up.

- Two flashing arrows indicate the exact point of protocol execution.
- Throughout the execution of the program, lid condition is constantly monitored, and when open, an intermittent “beep” sound will be audible and the message "NOT LATCHED LID” will be displayed. In this situation, voltage on strip is cut-off and the plate temperature is no longer controlled. To resume the current program running you should close the lid and press START button.
- When a protocol is running the display shows alternately a row containing the program identification number and name, a row that shows the instantaneous values of voltage (VOLT) and current per strip ("CURRENT") and a line which shows the cumulative value of volts / time allocated since the begin of a protocol.

PROTOCOL PAUSE AND STOP

When a protocol is running, you can pause the system by pressing the STOP / PAUSE. In this situation, voltage on strip is cut-off and the plate temperature is controlled.
When pause a protocol, it displays the word "PAUSE".
To exit the pause and resume the execution of the program, press START button, but if you press another time the STOP / PAUSE, the program end.

CURRENT CONTROL

When a protocol is running an automated check is constantly made to the current. The maximum current allowed through the strip is given by the product of the number of strip (STRIP N°.) and the value of current per strip (CURRENT/STRIP).When system detects that the amount of current flowing through the strip is greater than the desired maximum current, the applied voltage is decreased to the value that satisfies the constraint of current:

Current that crosses the Strip ≤ Strip Current * Number of Strip

See figure 11 for example.

The voltage limitation due to the current control is highlighted by the flashing of an asterisk "*" written next to "VOLT".

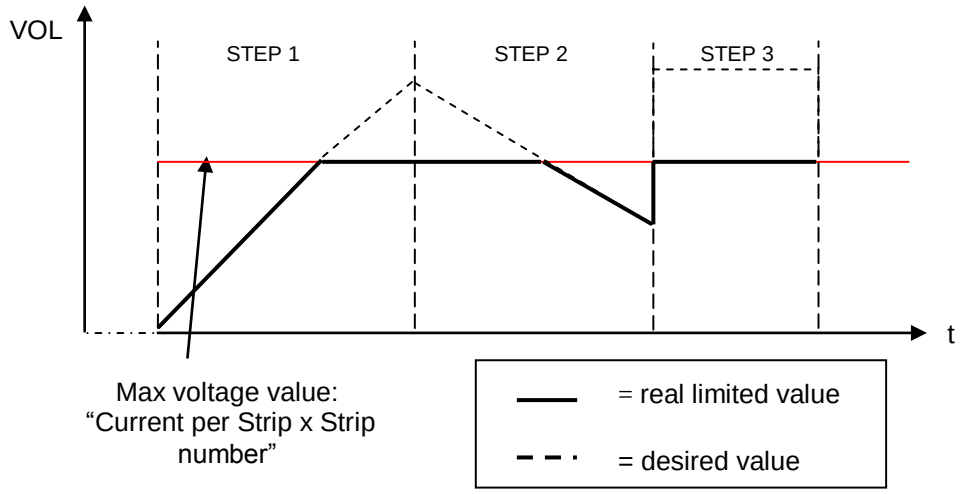


Figure 11

PROTOCOL EXECUTION END DATA (STATISTICS)

- The end of protocol is indicated by a sequence of “three beeps”, display will show "RUN COMPLETED" and LED will become red.
- By Pressing one of the two selection keys you will see the protocol "STATISTICS".

  Here the system will report the following data:

  - Identification program number
  - Name of the program
  - Rehydration phase total lenght;
  - Number of Strip
  - VOLT/hour for each step
  - Total time for each step
  - The sum of the values VOLT/hour of all the steps that are included in the protocol. The voltage applied during rehydration phase is not considered.
  - The total time of all STEP

To viewing over STEP 1 /5 and STEP 6/10 press UP and DOWN keys.

To exit from “STATISTICS “and return to the Main Menu, press one of two push buttons (+ / ENTER or - / ENTER).

5.6.2 Program Setup

In this section you can define up to 10 different programs. All data and parameters set written in this section are saved in memory.

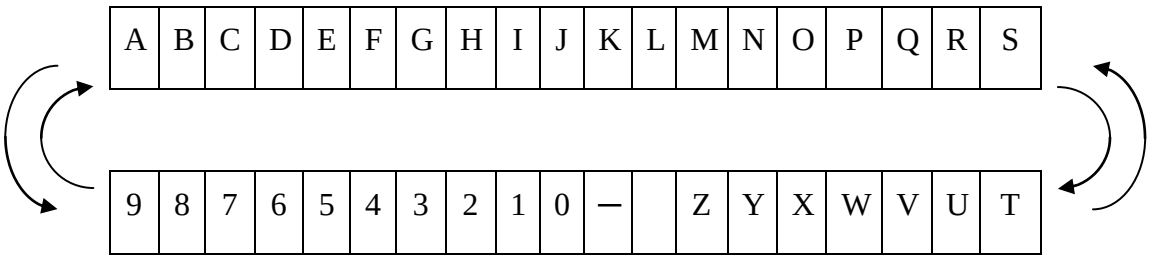
MAIN FEATURES OF PROGRAMS

- Each program is characterized by an identity number "PROG. NR." (from 1 to 10).
- It is possible to associate a name to each program (maximum length: 16 characters).
- Each program contains the data of a protocol with a maximum of 10 STEP. it is possible to choose whether the protocol should be preceded or not by the rehydration phase (according to the features planned in the SETUP).
- Each step is composed by a voltage (VOLT), a duration (TIME) and an operative mode (MODE) values.
- The total duration (TOTAL TIME) is calculated how the sum of any individual step length (included in the protocol) and the length of the rehydration phase (if included).

PROGRAM NAME

When cursor is moved inside the rectangle containing the name of the program, it is possible to change individually any of the 16 letters that compose the name.

Pressing the + and ─ let you scroll in a loop the following character sets:



REHYDRATION PHASE

A protocol may be preceded or not by the rehydration phase.

Move the cursor next to the written REHIDRATION and select YES to include it, NO to exclude this phase.

STEP SET-UP

Parameter	Range set	Measure Unit / Simbol
VOLT – step voltage	10 ÷ 15000 (*)	[V]
TIME – step lenght	MIN: 00:00 (**) MAX: 99:59	[hh:mm]
MODE – operative mode	STEP-AND-HOLD	
	GRADIENT	

(*) See “PROTOCOL END STEP” below

(**) See “JUMP OF A STEP” below

Note: If a protocol is programmed with less than 10 STEP, the cursor can be positioned on the first STEP zero volts, but not to the next step (see “PROTOCOL END STEP”). For example, if step 4 is the first null-step, is not possible to see next 6/10STEP.

STEP OPERATIVE MODE

Each step can be programmed in 2 different mode:

- **STEP-AND-HOLD**

  During the execution of a protocol, when a STEP programmed in this mode become active, the voltage applied to the strip is brought quickly to the value set in the appropriate field and is maintained for the duration of the STEP.

- **GRADIENT**

During the execution of a protocol, when a STEP programmed is this mode become active, the voltage applied to the strip is carried gradually from the initial VOLT value to the desired VOLT value set in the appropriate field.

The system is designed to work with increase and decrease operative mode (figure 12, an illustrative example).

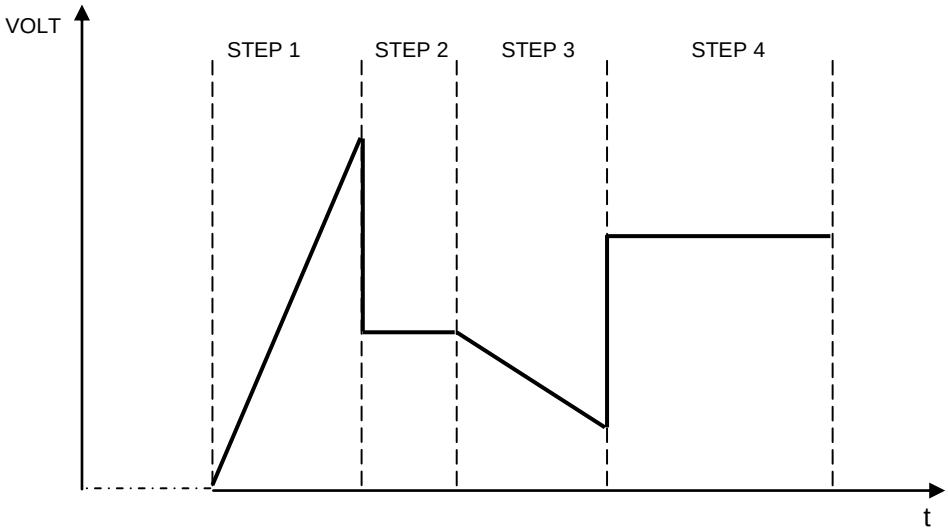


Figure 12

PROTOCOL END STEP

If a VOLT value is set to zero, all the parameters associated with the STEP and subsequent periods are replaced by symbol "- -". This STEP is the first null-step of the protocol and indicates the end of it.

In figure 13, the last step of the protocol is STEP 3

JUMP OF A STEP

If the value of TIME STEP is 00:00 , it means that that STEP will be skipped during the protocol execution.

In Figure 13, at the end of step 1, STEP 3 will start

STEP	VOLT [V]	TIME [HH:MM]	MODE
1	5000	10:00	
2	12000	00:00	– –
3	7000	5:40	
4	– –	– –:– –	– –
5	– –	– –:– –	– –

Figure 13

5.6.3 Setup

Within this section you can set parameters related to the rehydration phase and other general parameters. The parameters modified in this section are written in memory and are used in all next work sessions.

A. REHIDRATION PARAMETERS

Parameter	Range	Measuring unit
DURATION – rehydration lenght	MIN: 00:00 MAX: 99:59	[hh:mm]
TEMPERATURE – rehydration temperature	MIN: 15 MAX: 30	[°C]
VOLTAGE – rehydration voltage applied to the strip	MIN: 0 MAX: 100	[V]

B. GENERAL IEF PARAMETERS

Parameter	Range	Measuring unit
TEMPERATURE – protocol temperature	MIN: 15 MAX: 30	[°C]
CURRENT/STRIP – max current per strip	MIN: 10 MAX: 200	[μA]

5.7 Low voltage power supply (for EWS-1 only)

5.7.1 Initial informations

Using the low voltage power supply, thermostatic control of the plate is not provided. Moreover, is not necessary to use the protective cover.

All setting parameters are stored in the system memory. When you turn on the system when you log on to the "POWER SUPPLY" application, the parameters set during the last session are loaded. The bottom row of the display is the status bar and shows the current status of the device ("POWER SUPPLY STATUS"):

1. STOP
2. PAUSE
3. RUN

During stop and pause, the power is off (red LED). When RUN, the power is on (green LED illuminated).

5.7.2 Parameters Setting

Setting of parameters is allowed only when the power supply is not working ("STOP"). With the cursor keys and the "+" and "-" it is possible to choose and modify and one of the four parameters and manage the operation of the power supply.

- "MINIMUM CURRENT CONTROL": control over the minimum current output

  "NO" → no control over the current minimum is made;

  "YES" → a control is made on the minimum output current.

  During protocol execution, if current results below the minimum threshold (see next chapter on electrical characteristics and general), the power is turned off and an error message will appear together with a warning acoustic sound.

- "CONTROL TYPE": select the control mode. When this parameter is changed, the value and measuring unit are updated automatically.

During system working is ever performed voltage control to the connectors and the load current order to not exceed the max output values allowed by the system.

"VOLT" → voltage control is selected and active, the system acts on the value of output voltage, in order to ensure the voltage value set as a parameter.

"CURRENT" → current control is selected and active, the system acts on the output voltage to achieve and maintain a current output equal to the desired value.

"POWER" → power control is selected and active, the system acts on the value of output voltage and monitoring the load current, trying to achieve and ensure the power value set as a parameter.

- "VALUE SET": Based on the type of control chosen, you can set its parameter number within the specified range (see the next chapter on electrical characteristics and general).

- "TIME": time management of the device. If time value is "00:00", there is no countdown set. In this case all the operations must be stopped manually until the user decides to interrupt the session by pressing "STOP" button. If the value time is different than "00:00", all the operations will stop automatically once timer finish the countdown and supply will turn off.

5.7.3 Start and pause

After setting the desired parameters, press "START" for a few seconds ("RUN" will appear in the status bar). "REAL VALUES" table will indicate actual values of voltage, current and power. It is possible to "PAUSE " the protocol at any time by pressing "STOP button".

To resume, press "START ", the power is turned on again and system will re-start working. If you used timer mode, you can always stop manually the operations.

5.8 Error messages

In case of faulty errors an audible and visual alarm will appear.

Error code	Description	Solution
01	EEPROM error: memory problem.	Contact your local distributor or the manufacturer.
02	Temperature error: temperature system problem.	Contact your local distributor or the manufacturer.
03	Error on the voltage value measured by high-voltage module (IEFSYSTEM). Without any protocol running, is measured a voltage different from zero.	Contact your local distributor or the manufacturer.
04	Error on the voltage value measured by high-voltage module (IEF SYSTEM). During the execution of a program, the voltage applied to the strip is zero and cannot reach the set value.	Contact your local distributor or the manufacturer.
05	Current Error on the IEF SYSTEM module current. The current that pass through the strip has exceeded the maximum output allowed by the device.	Turn off the device, wait some seconds and then turn on again. Check the following parameters: current strip, voltage step, number of strip set. Try to run again the program. If the error recurs, contact your local distributor or the manufacturer.
06	Error on low voltage module (POWERSUPPLY). Without any protocol running, is measured a voltage different from zero.	Contact your local distributor or the manufacturer.
07	Error on the voltage value measured by low-voltage module (POWER SUPPLY). During the execution of a program, the voltage applied to the strip is zero and cannot reach the set value.	Contact your local distributor or the manufacturer.

08	Current Error on the POWER SUPPLY module current. The current absorbed has exceeded the maximum current allowed by the device.	Turn off the device, wait some seconds and then turn on again. Check if any short-circuit are present or check if the load has a very low impedance. Try to reactivate the power supply. If the error recurs, contact your local distributor or the manufacturer.

6. Troubleshooting

Problem	Probable reason	Suggested solution
The current is zero.	Incomplete contact between the electrodes and the paper bridge; incomplete contact between the paper bridge and the IPG strip; the lid is not properly closed.	Make sure that the IPG strip, the electrodes and the paper bridges are placed correctly; the IPG strip is not completely rehydrated: check the rehydration times and volumes; check that the lid is correctly closed.
The voltage is not increasing during the IEF.	The salt concentration of the sample is too high.	Desalt the sample; replace the paper bridges.
The maximum voltage is reached very slowly.	The programmed voltage is too high for the strip length; the salt concentration of the sample is too high.	We recommend to not exceed a voltage gradient of 330 V/cm; desalt the sample; replace the paper bridges after 2 hours of IEF.
Burning of IPG strip.	The current limit is too high; the strip has dried out; paper bridges are too wet; wrong paper bridge solutions.	We recommend a current limit of 50 µA per strip; make sure that the strip is completely covered by the Cover Fluid; make sure that the paper bridges are only wet, not saturated or dripping; use deionized water only.
Formation of urea crystals on the IPG gel surface.	Temperature of cooling plate too low.	Set the temperature at 20°C.
IPG strip turn white during IEF.	The strip has dried out.	Make sure that the strip is completely covered by the Cover Fluid.
Current does not drop during the IEF initial stage.	Wrong orientation of the strip (acidic end towards cathode); salt concentration of the sample is too high.	Check that the strip orientation is correct; desalt the sample; replace the paper bridges.

7. Recipes

Table 1: IPG strip rehydration stock solution*

(8 M urea, 2% CHAPS, 0.5/2% Ampholyte Buffer, 0.002% bromophenol blue)

Store in aliquots at -20 °C.

	Final concentration	**Amount**
Urea (FW 60.06)	8 M	12 g
CHAPS‡	2% (w/v)	0.5 g
Ampholyte Buffer (same range as the IPG strip)	0.5% (v/v)	125 µl
1% Bromophenol blue stock solution	0.002%	50 µl
Double-distilled water	—	to 25 ml

Table 2. Thiourea rehydration stock solution*

(7 M urea,2 M thiourea, 2% CHAPS, 0.5/2% Pharmalyte or IPG Buffer, 0.002% bromophenol blue)

Store in aliquots at -20 °C.

	Final concentration	**Amount**
Urea (FW 60.06)	7 M	10.5 g
Thiourea (FW 76.12)	2 M	3.8 g
CHAPS‡	2% (w/v)	0.5 g
Ampholyte Buffer (same range as the IPG strip)	0.5% (v/v)	125 µl
1% Bromophenol blue stock solution	0.002%	50 µl
Double-distilled water	—	to 25 ml

* DTT is added just prior to use: 7 mg DTT per 2.5-ml aliquot of rehydration stock solution.

‡ Other neutral or zwitterionic detergents (e.g. Triton X-100, NP-40) may be used at concentrations up to 2% (w/v).

Note: For the solubilization of more hydrophobic proteins it is recommended to use the urea/thiourea buffer instead of the urea buffer.

8. Technical specifications

HIGH VOLTAGE POWER SUPPLY

Power supply	100-250V, 50-60Hz
Max voltage output	15 KV
Power output	30 W
Voltage range	0 ÷ 15 KV
Current/strip range	10 ÷ 200 µA
Step timer range	00:00 ÷ 99:59 sec.
N. max strip	12
Volatge resolution	10 V
Storage protocols memory	10
Current resolution	1 µA

LOW VOLTAGE POWER SUPPLY (for EWS-1 only)

Power supplì	100-250V, 50-60Hz
Max voltage output	250V
Current range	10 ÷ 450mA
Timer	00:00 ÷ 99:59
Voltage resolution	10 V
Current resolution	1 mA
Operative mode	1) constant voltage 2) constant current 3) constant power

OTHER PARAMETERS

Working temperature range	10 ÷ 30 °C
Max humidity	< 90 % not condensing

9. Ordering information

PRODUCT CODE	DESCRIPTION
PRO-0012	EWS-1 Electrophoresis WorkStation
PRO-0010	P-Focus IEF system
PRO-0050	IEF strip tray (6 places)
PRO-0052	IEF strip tray (12 places)
PRO-0060	IEF equilibration tray for 8cm lenght strip – 14 places
PRO-0062	IEF equilibration tray for 24cm lenght strip – 6 places
PRO-0064	IEF equilibration tray for 45cm lenght strip – 6 places
PRO-0070	IEF rehydration tray for 8cm lenght strip – 14 places
PRO-0072	IEF rehydration tray for 24cm lenght strip – 6 places
PRO-0074	IEF rehydration tray for 45cm lenght strip – 6 places
PRO-0078	IEF Precut rectangular paper bridges (50pz)
PRO-0080	Cover Fluid
PRO-47712	IPG strip 4-7 / 7cm (1,5mm thickness) – 12 strips
PRO-471112	IPG strip 4-7 / 11cm (1,5mm thickness) – 12 strips
PRO-472412	IPG strip 4-7 / 24cm (1,5mm thickness) – 12 strips
PRO-310712	IPG strip 3-10 / 7cm (1,5mm thickness) – 12 strips
PRO-310NL712	IPG strip 3-10 NL / 7cm (1,5mm thickness) – 12 strips
PRO-610712	IPG strip 6-10 / 7cm (1,5mm thickness) – 12 strips
PRO-58712	IPG strip 5-8 / 7cm (1,5mm thickness) – 12 strips
PRO-36712	IPG strip 3-6 / 7cm (1,5mm thickness) – 12 strips
PRO-3101112	IPG strip 3-10 / 11cm (1,5mm thickness) – 12 strips
PRO-310NL1112	IPG strip 3-10 NL / 11cm (1,5mm thickness) – 12 strips
PRO-6101112	IPG strip 6-10 / 11cm (1,5mm thickness) – 12 strips
PRO-581112	IPG strip 5-8 / 11cm (1,5mm thickness) – 12 strips
PRO-361112	IPG strip 3-6 / 11cm (1,5mm thickness) – 12 strips
PRO-3102412	IPG strip 3-10 / 24cm (1,5mm thickness) – 12 strips
PRO-310NL2412	IPG strip 3-10 NL / 24cm (1,5mm thickness) – 12 strips
PRO-6102412	IPG strip 6-10 / 24cm (1,5mm thickness) – 12 strips
PRO-582412	IPG strip 5-8 / 24cm (1,5mm thickness) – 12 strips
PRO-362412	IPG strip 3-6 / 24cm (1,5mm thickness) – 12 strips

PRO-URE	Urea MB grade 500g
PRO-THI	Thiourea 99% 500g
PRO-AMB	Ampholyte Buffer
PRO-SER5810	SERVALYT™ 5-8; 10ml
PRO-SER5825	SERVALYT™ 5-8; 25ml
PRO-SER582	SERVALYT™ 5-8; 2ml
PRO-SER6910	SERVALYT™ 6-9; 10ml
PRO-SER6925	SERVALYT™ 6-9; 25ml
PRO-SER692	SERVALYT™ 6-9; 2ml
PRO-SER31010	SERVALYT™ 3-10; 10ml
PRO-SER31025	SERVALYT™ 3-10; 25ml
PRO-SER3102	SERVALYT™ 3-10; 2ml
PRO-SER4710	SERVALYT™ 4-7; 10ml
PRO-SER4725	SERVALYT™ 4-7; 25ml
PRO-SER472	SERVALYT™ 4-7; 2ml
PRO-CHP25	CHPAS 25 gr
PRO-CHP100	CHPAS 100 gr
PRO-DTT	DTT (dithiothreitol) MB grade 5g
PRO-IAA5	IAA (iodoacetamide) 5g
PRO-IAA25	IAA (iodoacetamide) 25g
PRO-TRX	Triton X-100 MB grade 250ml
PRO-TRI	Tris MB grade 1 Kg.
PRO-GLY	Glycine analytical grade 1 Kg.
PRO-SDS	SDS (sodium dodecylsulphate) MB grade 500g
PRO-MWM	Molecular Weight Markers Proteome markers 1 kit (5vials)
PRO-AGA	Agarose for DNA electrophoresis 500g
PRO-GLE	Glycerol MB grade 1L
PRO-BRB	Bromophenol Blue 25g

10. References

- Cargile BJ, Bundy JL, Freeman TW, Stephenson JL Jr (2004). Gel based isoelectric focusing of peptides and the utility of isoelectric point in protein identification. J Proteome Res 3(1):112–119.
- Cargile BJ, Sevinsky JR, Essader AS, Stephenson JL Jr, Bundy JL (2005). Immobilized pH gradient isoelectric focusing as a first-dimension separation in shotgun proteomics. J Biomol Tech 16 (3), 181-9.
- Eriksson H, Lengqvist J, Hedlund J, Uhlen K, Orre LM, Bjellqvist B, Persson B, Lehtio J, Jakobsson PJ (2008). Quantitative membrane proteomics applying narrow range peptide isoelectric focusing for studies of small cell lung cancer resistance mechanisms. Proteomics 8(15):3008–3018.
- Fraterman S, Zeiger U, Khurana TS, Rubinstein NA, Wilm M (2007). Combination of peptide OFFGEL fractionation and label-free quantitation facilitated proteomics profiling of extraocular muscle. Proteomics 7(18):3404–3416.
- Geiser L, Vaezzadeh AR, Deshusses JM, Hochstrasser DF (2011). Shotgun proteomics: a qualitative approach applying isoelectric focusing on immobilized pH gradient and LC-MS/MS. Methods Mol Biol 681, 449-58.
- Heller M, Michel PE, Morier P, Crettaz D, Wenz C, Tissot JD, Reymond F, Rossier JS (2005). Two-stage Off-Gel isoelectric focusing: protein followed by peptide fractionation and application to proteome analysis of human plasma. Electrophoresis 26(6):1174–1188.
- Millioni R, Miuzzo M, Puricelli L, Iori E, Sbrignadello S, Dosselli R, Cecconi D, Tessari P, Righetti PG (2010). Improved instrumentation for large-size two-dimensional protein maps. Electrophoresis 31(23-24):3863-6.
- Poznanovic S, Schwall G, Zengerling H, Cahill MA (2005). Isoelectric focusing in serial immobilized pH gradient gels to improve protein separation in proteomic analysis. Electrophoresis 26(16):3185-90.
- Xiao Z, Conrads TP, Lucas DA, Janini GM, Schaefer CF, Buetow KH, Issaq HJ, Veenstra TD (2004). Direct ampholyte-free liquid-phase isoelectric peptide focusing: application to the human serum proteome. Electrophoresis 25(1):128-33.