A Geno Technology, Inc. (USA) brand name

Semi-dry Electrotransfer Unit

Cat. No. BT302

1-800-628-7730 ♦ 1-314-991-6034 ♦ info@BTLabSystems.com

Before using the instrument, please read the operation instruction handbook carefully. We recommend that all the components and accessories be cleaned with a suitable laboratory cleaner, and rinsed thoroughly with distilled water before use.

INTRODUCTION

BT Lab Systems' BT302 Semi-dry Electrotransfer Unit is used together with electrophoresis power supply for transferring the proteins in the polyacrylamide gels onto the nitrocellulose membrane, nylon membrane or PVDF membrane.

Semi-dry blotting is performed with graphite plate electrodes in a horizontal configuration, sandwiching a gel and membrane between sheets of buffer-soaked filter paper that function as the ion reservoir. During the electrophoretic transfer, negatively charged molecules migrate out of the gel and move towards the positive electrode, where they are deposited on the membrane. The plate electrodes, separated only by the gel and filter paper stack, provide high field strength (V/cm) across the gel, carrying out the very efficient, rapid transfers.

The transfer surface of the BT302 is 200 × 200 (mm), suitable for transferring large format gels.

BT302 Semi-dry Electrotransfer Unit is a semi-dry blotting system designed for fast set-up and easy to use.

PRODUCT OVERVIEW

The Semi-dry Electrotransfer Unit consists of the following parts: main tank body, lid, lead,spacing piece. Please refer to figure (1) for part identification.

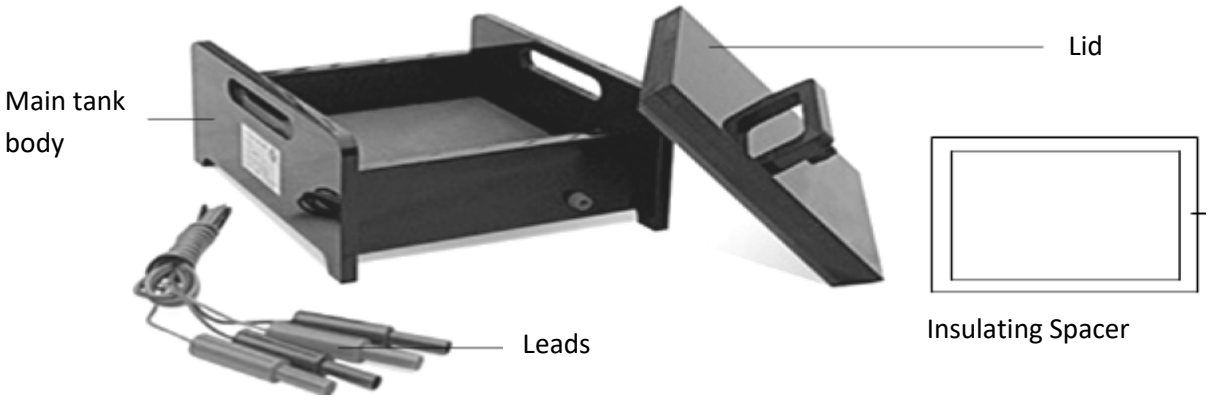

Figure 1

TECHNICAL SPECIFICATIONS

Transfer area: 200 x 200 (mm)
Max input voltage: 600V
Continuous working time: 24 hours
Dimensions: 270 x 250 x 104 (mm)
Weight: 4 kg

OPERATING INSTRUCTIONS

Important tips

Do not connect the instrument with the power supply before starting the experiment. Electric current to the instrument, provided from the instrument power supply, enters the unit through the lead and lid assembly, providing a safety interlock to the user. Electric current to the instrument is broken when the lid is opened and removed. Please don't attempt to use the instrument without the lid, and remember to turn the instrument power supply off before opening and removing the lid.

Working conditions:

The instrument is intended for indoor use only; Ambient temperature: 0- 40°C (32 - 104°F); relative humidity <80%; There should be no strong vibration around the instrument and the platform should be level and smooth.

To transfer proteins, prepare the electrophoresis cell, assemble the transfer stack and connect to an electrophoresis power supply. Then run the transfer for the required amount of time. The steps are as follows:

Choice of immobilizing material:

Types of membranes: There are three types of blotting membranes: nitrocellulose, nylon and polyvinylidene fluoride (PVDF).

1. **Nitrocellulose membrane** – The nitrocellulose membranes are the most popular membranes for Western Southern and Northern blotting. The nitrocellulose membrane has good binding capacity, proteins bind to the membrane due to hydrophobic interactions. The features of nitrocellulose membrane are determined by its pore diameter and its components. Its ability of combination is in contrast with/to its pore diameter. At the early stage of using the instrument, we suggest you use 0.45µm of membrane. Do not use nitrocellulose membrane containing cellulose acetate.
2. **PVDF membrane** – PVDF membranes have higher binding capacity than nitrocellulose and it has a strong hydrophobic character and is solvent resistant. It is physically stronger than nitrocellulose membrane and it is compatible with commonly used protein stains and immunodetection methods.
3. **Nylon membrane** – Nylon membranes are microporous, positively charged, ultra-pure nylon, bound to a polyester support. It is ideal for binding negatively charged biomolecules such as DNA and RNA.

   Note: The nylon membranes also have a higher binding capacity with the negatively charged dye like Amino Black and Commassie Blue.

Filter paper for the experiment: We suggest you use 3MM filter paper that will help you to get better experiment results.

Buffer preparation:

The compounding method of reagent for transferring protein from the SDS gel.

1. Continuous buffer system

   Glycine, 39mmol/L; Tris, 48mmol/L; SDS, 0.0375% (w/v); 20% methanol (v/v), these liquids (reagents) all need to be mixed with double distilled water. These buffer solutions can be used as anode buffer solution, and can be used as cathode buffer solution, too.

2. Non-continuous buffer system

   Anode buffer I: 0.3mol/L Tris, pH10.4, 20% (v/v) methanol. Anode buffer II: 25mmol Tris, PH10.4, 20% (v/v) methanol. Cathode buffer: 4mmol/L; 6-Amino-n-acetic acid, pH7.6, 20% (v/v) methanol. Recommended parameters: Electric current: 0.8mA— 1mA /cm^2, time:0.5—1 hour.

The compounding method of reagent for transferring protein from the Iso-electric focusing (IEF) gel.

1. Continuous buffer system: It is the same as that of SDS gel
2. Non-continuous buffer system
   Anode buffer I: 0.3mol/L Tris, pH10.4
   Anode buffer II: 0.1mol/L Tris, pH10.4
   Cathode buffer: 0.1mol/L arginine (Arg), 0.01%（w/v） SDS, pH10.5.
   Recommended parameters: Electric current: 0.8mA—1mA /cm^2, time:0.5—1 hour.

The compounding method of reagent for transferring protein from the agarose gel.

Non-continuous buffer system
Anode buffer I: 0.3mol/L Tris, pH10.4.
Anode buffer II: 25mmol Tris pH10.4.
Cathode buffer: 40mmol/L 6-Amino-n-acetic acid, pH7.6.
Recommended parameters: Electric current: 0.8mA—1mA /cm^2, time: 1/2 of that of PAGE gel.

Preparation of the transfer stack

Note: Wear gloves to avoid contamination of the membranes

1. Prepare the gel (1 pc), nitrocellulose membrane (1 pc), 3mm filter paper (6 pcs), cut the nitrocellulose membrane and the six pieces of filter paper to the same size as the gel.
   Note: Cut the membrane and the filter papers the same size as the gel or slightly smaller.
2. Steep the nitrocellulose membrane and the filter paper into the buffer solution for about 30 minutes.
3. Place the Insulating spacer into the main tank body.

   Note: Rinse the electrophoresis cell with distilled water before you use it.

4. Put 3 pieces of the overlapped filter paper into the main tank body.

Note: a) The filter papers steeped in the buffer solution should have less buffer solution when they were taken out;

b) If you use 3MM filter paper, only one layer of filter paper is enough.

5. Then place the gel on it (Proteins bind to the membrane as soon as contact occurs, so it is important to place the gel correctly on the first try) and put the nitrocellulose membrane again, and finally, put the last 3 pieces of overlapped filter paper on this membrane. Please refer to figure 2.

   Note: The lowest 3 filter papers should not contact the top 3 ones. If they contact each other, this will cause a short circuit.

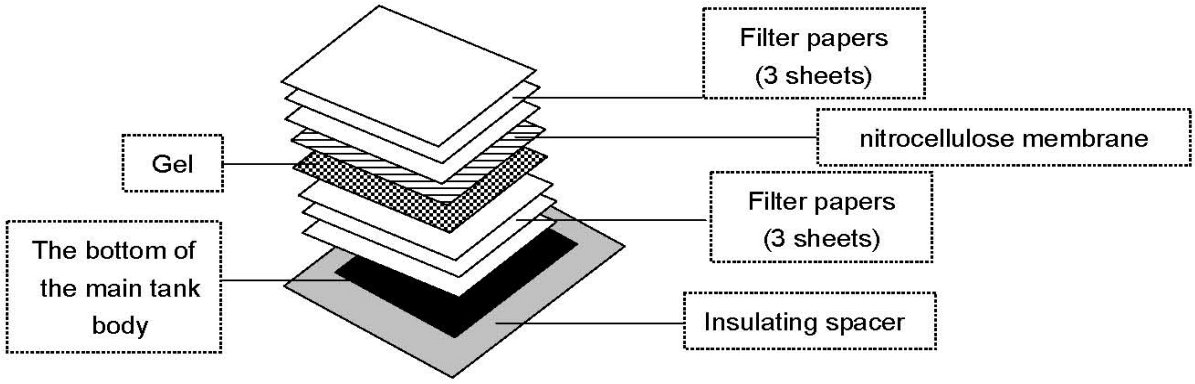

Figure 2

6. Extrude the bubbles with a clean glass stick from the center to the edges after putting the filter paper, the gel and the nitrocellulose membrane. You can add a few drops of buffer to trouble areas to help extrude the air bubbles. Please refer to figure 3.

Figure 3

Start transferring

1. Cover the lid with the positive electrode.
2. Connect the electrophoresis cell with the electrophoresis power supply, matching red lead to red output terminal and black lead to black output terminal. Do not reverse the polarity, this will result in defeat of the experiment. The lid contains the red, or positive electrode, the main tank body contains the black, or the negative electrode, choose the proper parameters according to the gel size. Then turn on the "ON/OFF" switch of the power supply and start running, the gel will be transferred for about 0.5 – 1 hours at a constant current (1 – 2mA/cm_2).

   Note: Please don't remove the lid of the electrophoresis cell until after the transfer is complete in order to prevent stack components from moving.

After transferring is completed

1. Turn off the electrophoresis power supply.
2. Disconnect the leads from the power supply.
3. Remove the lid carefully and slowly.
4. Remove and dispose of the upper blot filter papers.
5. Peel off the gel from the nitrocellulose membrane carefully, the transfer efficiency can be monitored by staining the gel with Coomassie blue R-250 protein stain or with silver stain.
6. Remove the membrane from the stack with blunt forceps.
7. Remove the remaining blot filter papers and dispose of them.
8. Rinse the electrophoresis cell and dry it for the next use.

   Note: When you rinse the electrophoresis cell, don't splash liquid into the lead socket.

Attention

The main tank body and the lid of the instrument are very fragile, do not bump or drop it during the course of packing, transportation and experiments, especially when you clean the electrophoresis cell.

MAINTENANCE

The product should be stored under the following conditions ambient temperature: - 40°C (-0°F)～55°C (131°F) ; the relative humidity: ≤93% ； no corrosive gas; not in a drafty area. Please clean the electrophoresis tank after you are finished. You can clean it with a sponge. Rinse the main tank body (buffer tank) thoroughly with distilled water after every use. Then wash it with deionized water. Air-dry it for the next use.

Note: When you rinse the electrophoresis cell, please don't splash the liquid into the lead socket.

Keep the electrophoresis cell at a distance from the electrophoresis power supply lest the power supply should be damaged by the splashing liquid when you pour or release the solution.

WARRANTY

The instrument is warranted against defects in materials and workmanship for 1 year. If any defects occur in the instrument or accessories during this warranty period, BT Lab Systems will repair or replace the defective parts at its discretion without charge.

For any inquiry or request for repair service, contact your local BT Lab Systems office. Inform BT Lab Systems of the model and serial number of your instrument.

TECHNICAL SUPPORT

BT Lab Systems offers technical support for all of its products. If you have any questions about the product's use or, operation, please contact BT Lab Systems at the following info.

E-Mail: info@BTLabSystems.com