



English

STARLAB Semi-dry Electro-Blotter Mini 10 x 10cm

Catalogue No. N2011-1110

STARLAB Semi-dry Electro-Blotter Maxi 20 x 20cm

Catalogue No. N2021-2110

Please read the entire operators manual thoroughly before operating this unit! These units are capable of delivering potentially lethal voltage when connected to a power supply and are to be operated only by qualified technically trained personnel.

Warranty

Please check that the unit has been received complete and undamaged. Refer to the illustration below and to Table A to check that all components are present. Save all packaging and documents until you have thoroughly inspected your shipment; if you find that your order is incorrect or damaged, call STARLAB for instructions for its return.

This warranty is valid for 36 months, only if the product has been used and cared for according to this User Manual. No liability is accepted for loss or damage arising from incorrect use. The liability is limited to the repair or replacement of the unit, or a refund of the purchase price, at STARLAB's option. STARLAB is not liable for any consequential damages. We refer to our general terms for sale.

STARLAB reserves the right to alter the specifications of the Blotting Systems without prior notice. This will enable us to implement improvements as soon as they are available..



STARLAB Semi-dry Electro-Blotter System components:

TABLE A: System components included

TABLE A: Included System Components		
	N2011-1110	N2021-2110
Blotting Unit	1	1
Safety Lid, with attached power cords	1	1
Wing Knobs, black	3	3

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I. ENVIRONMENTAL CONDITIONS FOR USE

- This unit is intended for indoor use only.
- This unit can be operated safely at an altitude of 2,000m.
- The normal operating temperature range is between 4°C and 65°C.
- Maximum relative humidity 80% for temperatures up to 31°C, decreasing linearly to 50%, relative humidity at 40°C

II. SAFETY PRECAUTIONS

Please read the User Manual carefully before using the STARLAB Long Horizontal Electrophoresis Unit. This manual contains important operating and safety information. STARLAB`s Blotting units are designed to perform flawlessly for years in the most demanding laboratories. Please take the time to read this manual so you understand the safety and operating instructions to ensure the successful use of the unit. Alterations could cause serious injury to the user or the system.

Power to the unit is supplied by an external power supply. The power supply must meet safety standards for IEC 1010-1 regulations and must be ground isolated and incorporate a no-load detecting circuit.

Power is supplied to the gel through the cover of the system providing a safety interlock to the user. Users should not attempt to operate this unit without the safety inter-locked cover in place.

- To avoid the risk of personal shock always disconnect the unit from the power supply before:
 - a) removing cover, b) moving the unit or, c) adding buffer
- Running Conditions should not exceed the maximum operating voltage or current
- Use this apparatus only for its intended purpose and as described in this manual.
- Do not use the product if the power cords are damaged or if its surfaces are cracked.

III. INTRODUCTION

The STARLAB Semi-Dry Electro-Blotter enables rapid and highly efficient transfer nucleic acid or protein fragments from polyacrylamide or agarose to blotting membranes such as nitrocellulose, nylon or PVDF membranes. Electro blotting of gels is more efficient than capillary methods – often taking less than an hour – and is suitable for “Southern”, northern or western blotting.

STARLAB Semi-Dry Electro blotter`s solid plate platinized titanium and stainless steel electrodes are highly conductive and allow transfer at low voltages without external cooling systems. The systems use large flat electrodes and consume significantly less transfer buffer without a loss in transfer efficiency or signal strength.

Outstanding features ensure trouble-free use

- Robust acrylic construction stands up to daily usage without breakage, warping or leakage.
- Gold Plated Electrodes, corrosion free and rated safe up to 1,000 volts
- Safety Cover with attached power cords assures safety and prevents reverse orientation of electric field
- All of STARLAB`s Electro Blotters are hand-made to provide unmatched durability for years of daily use.

Intelligent design results in exceptional resolution

- Conserve buffer and allow rapid transfer – choose from a Mini or Maxi configuration
- Large surface area of plate electrodes maximises transfer area to improve efficiency

Once proteins (nucleic acids) in a sample aliquot have been separated on a slab gel, the resulting bands may be transferred to a solid support membrane. The primary reason for this type of blot is one of localisation and secondarily, concentration of discrete protein bands. Although many have used alternative

cross linking agents such as DATD (N, N''-dihydroxyethylene-bis Acrylamide) to allow for the accessibility of gel bound proteins, this still represents an impediment to radio enumeration due to the quenching by the gel matrix itself.

The most common solid support membrane is nitrocellulose. A second type of membrane is PVDF (Polyvinylidene difluoride), which is generally used when a transferred protein is to be sequenced, additionally; it has a 2x binding capacity. Also used for nucleic acid capture are Nylon membranes. In either case, the proteins are transferred from the gel to the matrix in an electric field perpendicular to the gel (initial running direction).

Tris based buffers are employed in the transfer. Methanol and SDS are modifiers often used in protein transfer buffer. These components however are antithetical in their effects both in terms of movement and absorption. Methanol restricts protein movement from the gel but is often required to support the ionic nature of protein to nitrocellulose binding. SDS aids in protein elution, but can also inhibit binding of small molecular weight proteins (Mozdzanowski, J., High yield electroblotting, Electrophoresis, 1992, Vol 13., p.59-64).

IV. GENERAL CARE & CLEANING

WARNING!

Acrylic is not resistant to aromatic or halogenated hydrocarbons, ketones or esters. Organic solvents cause acrylic to "craze" or crack. Do not use ethanol or other organic solvents to clean the unit.

- Do not autoclave, bake or microwave your unit.
- Before using, clean and dry the unit. Clean with DISTILLED WATER ONLY; dry parts with clean, lint-free lab wipes or, preferably, air dry. Use care when cleaning or drying the unit near the platinum wire. The connectors should be clean and dry before usage or storage.
- Do not use abrasive creams or scourers.
- Do not use cleaning brushes in the electrode area.

- A thorough rinse with distilled water is all that is generally required to clean the unit after use. A mild detergent may also be used. Acrylic can also be exposed to a mild bleach solution (10:1). In addition RNase removal products are also safe for acrylic.

V. SETTING UP

Power Supply: Blotting requires a power supply than can operate at a fairly high current setting and low voltage. If an inappropriate power supply is used, the power supply may blow a fuse, shut itself off, display a no load or short load message or even have a short circuit. It is very important to be sure that the power supply you will be using will work with this device.

Blotting Buffer: The most commonly used buffer for protein blotting from polyacrylamide gels is Towbin buffer. Small amount of buffer may be needed for equilibrating the gel and membrane prior to blotting. The buffer should be cooled to 4°C.

Filter Paper: Large sheets or rolls can be cut and used.

Blotting Membrane: Nitrocellulose and PVDF (polyvinylidene difluoride) can be used for proteins; charged Nylon membranes can be used for nucleic acids. The choice depends on the user's preference and sometimes the detection method used.

Operating Instructions:

DNA/RNA: If these gels were not run in 1XTBE, they should be equilibrated for 10 minutes in this buffer.

Protein Gels: After electrophoresis, wearing gloves, remove the gel assembly from the apparatus and place it under cold, running water until the surfaces of both the glass plates are cool. This facilitates handling of the gel and prevents the gel from "curling".

- Place the gel assembly, with the notched or shorter plate facing up, flat on paper towels on the lab bench and remove any excess liquid.
- Remove one side spacer and insert a long metal spatula between the glass plates where the spacer had been. Use care not to insert the blade close to the notches. Gently rock the spatula, forcing separation of the plate from the gel. The gel will normally remain affixed to the bottom (non-notched) plate. Remove the top (notched) plate by slowly lifting it from the side with the inserted spatula and gradually increasing the angle until the plate is completely separated from the gel.

If the gel sticks to the top plate in an isolated spot, a stream of water from a squirt bottle can be sprayed at the spot of separation.

- Once the plates are separated, remove the second side spacer along with any extraneous bits of acrylamide around the gel. If excess water was utilized to aid in the separation of the gel from the glass, use a paper towel to absorb the excess liquid.
- Using a dry piece of dry blotting paper gently roll it onto the gel beginning at one end and working slowly towards the other end. Care should be taken to prevent air bubbles from forming between the paper and the gel.
- Beginning at one end of the gel, slowly lift and peel back the paper from the glass with the gel attached. As before, a stream of water from a squirt bottle may be used to aid separation of the gel from the glass plate.
- Place the gel in blotting buffer and agitate gently.
- Cut six (6) pieces of Extra Thick blotting paper and a piece of blotting membrane to the exact size of the gel to be blotted.
- Mark the membrane, to indicate the side to which the samples will be on. This is important in the event that any successive probe is negative, and to indicate sample orientation.

- Wet the membrane according to its manufacturers' recommendations, followed by a quick equilibration in transfer buffer.
- It is often helpful to have all the filter paper and membrane sitting in transfer buffer as you start to build the blotting sandwich.
- Lay three (3) pieces of thoroughly soaked filter paper on the bottom (anode) electrode plate of the blotting unit. Make sure that the pieces are lined up squarely. Using a roller and only gentle pressure remove any air bubbles from between the papers.
- Add a few ml of buffer to the papers and place the thoroughly soaked blotting membrane on top of the filters papers.
- Add a few mil of buffer to the membrane - remove the gel from the blotting buffer and place on top of the blotting membrane. Be careful not to introduce any air bubbles.
- Finally, place three (3) thoroughly soaked filter papers on top of the gel. Use a roller to gently remove any air bubbles from the stack.
- Holding the stack, drain off all excess buffer from the plate. Wipe away any droplets around the edges of the stack.

VI. RUNNING THE BLOT

- Place the lid on the unit and loosely tighten down the lid with the supplied black knobs. The weight of the lid is usually enough, and the tightening is not required.
Note: Do not tighten for gels thicker than 1.5mm, or when using more than six (6) filter pads total. If the screws are used, tip the unit on an angle, to drain off any excess buffer that may have been squeezed out. The buffer will remain safely in the moat around the electrode plate.

- Attach the power leads (red to red and black to black) to an appropriate power supply. The black lead has a acrylic shroud that will stop it from attaching to the red anode.
- Run the Blot

Transfer Settings

Blotting takes place at a given migration rate for a specified time. The units are mA times hrs. If you need to slow the transfer down, to say coincide with the setting up of a probe, simply decrease the current (mA) to match the added time you require.

$$(mA)(hr) \text{ Std Setting} = (mA)(hr) \text{ New Setting}$$

alternatively, you can increase the current to decrease the time. This assumes that you have determined an initial mAh value that works well for the molecules you are interested in.

A current to use for a 45 minute time period is based on the area (cm*cm) of your gel. The range is 0.8 to 2 mA per square centimetre of gel..

For example: the area of a 10 x 10 cm gel would be 100cm², the current range would be 80mA (0.8mAcm x 100cm²) to 200mA (2mAcm x 100cm²)

Blots may also be run at a constant voltage. Some power supplies have difficulty sustaining steady voltages at these low voltage settings. If you find that voltages are fluctuating, or that the power supply shuts itself off when set on constant voltage, use constant current settings instead. Read your power supply's instructions to ensure that the power supply will work at a voltage lower than 10V. These voltages often occur in semi-dry electroblotting. Contact the manufacturer regarding the unit's performance under high current, low voltage conditions if you have any questions.

Factors that affect transfer efficiency:

While general conditions can be described which will result in successful transfer of most molecules, it should be noted that optimal transfer conditions will vary based on the characteristics of the molecule you are working with. Some factors that affect transfer rate and efficiency include molecule size, charge, gel thickness and percentage, and hydrophobicity. The reference list at the end of the manual provides useful information that can help you choose optimal conditions for efficient transfer of a specific molecule.

	RUNNING CONDITIONS	
	Protein	DNA/RNA
Membrane	Nitrocellulose .45 or 0.2μ, PVDF 0.45μ or 0.2μ	Nylon
Transfer Buffer	Towbin Buffer, 3 Buffer System, Bjerrum and Schafer-Nielsen	0.5X – 1XTBE, TAE, NAQ
Power Settings	Constant current 0.8-3mA per cm ² gel surface area 10-14 Volts maximum	Constant Current 0.5 3mA/cm ² gel surface area 10-14 Volts maximum
Running Time	30 minutes to 2 hours Needs to be experimentally determined (large molecules need longer transfer time)	30 minutes to 2 hours, generally in the lower range

TECHNICAL INFORMATIONAL TIPS

Transfer Times

Transfer times must be determined experimentally. This is because transfer time is dependent upon:

- Percentage of gel
- Type and amount of cross linking in the gel
- Type of protein: cytoplasmic, membrane, nuclear
- Size of protein

There is no formula for determining transfer time. There are too many variables involved to give specific transfer conditions that will work for every protocol.

The guidelines are just a starting point – exact conditions must be determined

2mA/cm² of gel for 1 hour

Different Kinds of Blotting

Western Blotting: a blotting method for proteins that uses specific antibodies attached to a particular protein to help identify it. It is often performed after SDS-PAGE or some other form of polyacrylamide gel electrophore

“Southern” Blotting: a method sometimes called hybridization because a radioactive probe is “hybridized” or attached to specific pieces of DNA.

Northern Blotting: similar to “Southern” but the molecules involved are RNA

Both “Southern” and Northern blotting generally requires the DNA or RNA to first be separated out on an agarose gel.

VII. TROUBLESHOOTING

PROBLEM: Transfer Efficiency is Poor	
Cause: Current is too Low	Solution: Semi-Dry Transfer should be performed at constant current. Current density should be between 0.5 and 3mA/cm ² of stack surface area.
Cause: Power Supply is inappropriate	Solution: Many power supplies will

for Semi-Dry Transfer	shut off or blow a fuse when run at the conditions required for semi-dry transfer. Semi-dry transfer requires low voltage (often less than 10V) and high current. Check with the manufacturer of the power supply to determine whether it is appropriate for semi-dry transfer.
Cause: Transfer performed for too short a time	Solution: Increase the amount of time for transfer
Cause: Transfer sandwich was assembled in the wrong order	Solution: The Insight™ Semi-Dry Electroblotter is configured with the cathode on the top and the anode on the bottom. This means that a downward transfer is being performed. Follow instructions carefully when assembling the transfer sandwich.
Cause: The pH of the transfer buffer is too close to the isoelectric point of the protein	Solution: Try a more acidic or basic transfer buffer
Cause: Too much methanol in the transfer buffer	Solution: Reducing methanol can help elute proteins from the gel, but can reduce binding to nitrocellulose membranes.
Cause: High percentage gels restrict transfer	Solution: Higher percentage acrylamide or crosslinker can restrict elution of proteins. Use the lowest percentage acrylamide possible to separate your proteins.

Cause: Puddles of buffer were present on the anode, allowing the current to bypass the stack	Solution: Always clean the lower plate before placing the lid on the unit. Do not squeeze the stack excessively as this also creates puddles that the current can pass through.
Cause: The filter paper was too dry	Solution: Filter paper should be saturated with transfer buffer before adding them to the sandwich
NITROCELLULOSE MEMBRANES	
PROBLEM: Insufficient binding of proteins to the membrane	
Cause: Over transfer through the membrane	Solution: Use 0.2 micron pore size nitrocellulose instead of 0.45 micron, or use PVDF with a higher binding capacity
Cause: Not enough methanol in the transfer buffer	Solution: Nitrocellulose binds proteins best when 20% methanol is used in the transfer buffer
Cause: Low MW proteins are not binding well or are being washed away	Solution: Use glutaraldehyde to crosslink the proteins to the membrane and use Tween-20 in the wash steps
Cause: SDS is preventing binding	Solution: Eliminate SDS in the transfer buffer
PVDF	
PROBLEM: Smeared or swirled transfer and missing bands	

Cause: Membrane was dried out before it was added to the transfer sandwich	Solution: Membrane should be completely grey and slightly translucent when added to the sandwich. If it has dried out re-wet in methanol and equilibrate in transfer buffer
Cause: Alcohol was not used to pre-wet the membrane	Solution: PVDF is hydrophobic and requires a short soak in methanol prior to transfer
Cause: Air spaces are interfering with contact between the gel and the membrane	Solution: Roll a test tube or pipette over the membrane (make sure it is clean) before putting the rest of the sandwich together
Cause: Electrophoretic conditions were incorrect or not ideal	Solution: Running conditions, sample preparation, percentage acrylamide, and many other variables can affect the migration and resolution of proteins. Please review your electrophoretic conditions
Cause: Transferring at too high a current	Solution: Please refer to the Running Conditions in the manual
Cause: Membrane was not thoroughly wetted	Solution: Always pre-wet the membrane according to the manufacturer's instructions. White spots indicate dry areas of the membrane.
Cause: Too much Current	Solution: Running at constant voltage can cause power fluctuations that will cause overheating. A buffer that has not been made correctly or that has too high an ionic strength can also burn a gel by overheating. A cracked and dry gel often is an indicator of overheating