

PosiBlot® 30-30 Pressure Blotter and Pressure Control Station

INSTRUCTION MANUAL

Catalog #400330–400333

Revision #102002a

IN #70084-02

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PosiBlot® 30-30 Pressure Blotter and Pressure Control Station

MATERIALS PROVIDED

Material provided	Quantity			
	Catalog #400330	Catalog #400331 (120 V)	Catalog #400332 (230 V)	Catalog #400333 (100 V)
PosiBlot® 30-30 pressure blotter	1	1	1	1
Masks	5	5	5	5
Membrane support pad	1	1	1	1
Sponges	2	2	2	2
Pressure Control Station	—	1	1	1
Connector hose	—	1	1	1
Power cord	—	1	1	1

ADDITIONAL MATERIALS REQUIRED

Hybridization membrane
Whatman® 3MM paper
Agarose gel
Transfer buffer

Revision #102002a

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INTRODUCTION

The PosiBlot® 30-30 pressure blotter* is part of an integrated system designed to transfer DNA or RNA from agarose gels quickly and efficiently onto solid support matrices, such as Stratagene's hybridization membranes including the Nitrocellulose Membranes,† Duralose-UV™ membranes† (reinforced nitrocellulose), and Duralon-UV™ membranes† (nylon) (for chemiluminescent detection).

Note *The PosiBlot 30-30 system can only be used with Stratagene's Pressure Control Station.*

OPERATING INSTRUCTIONS

The following guidelines were determined using Stratagene's hybridization membranes. When compared to the standard capillary technique, the PosiBlot 30-30 pressure blotter will decrease transfer time from 12 hours to approximately 1 hour, while maintaining excellent resolution and sensitivity. Perform the procedures at room temperature.

Gels with <1% agarose, which are 0.5 cm thick or less, produce optimal results. If possible, avoid loading the samples in the first and last lane. This makes it easier to cut the proper size mask. While running the gel or during the gel treatment steps, prepare the mask, the sponge and the membrane.

Pretreating the Gels

Pretreating the Gels Prior to Southern Transfer

1. Following electrophoresis, stain the gel in 5 µg/ml of ethidium bromide (EtBr) in water. Destain the gel in water and photograph it.
2. Depurinate the gel according to the appropriate protocol:

Vertical Gels (1.5- or 3-mm spacer)

Treat the gels with 0.25 N HCl for 5 minutes with gentle shaking. The HCl solution should cover the gel completely.

Horizontal Gels (5-mm spacer)

Treat the gels with 0.25 N HCl for 15 minutes with gentle shaking.

Horizontal Gels (10-mm spacer)

Treat the gels with 0.25 N HCl for 30 minutes with gentle shaking.

Note *The bromophenol blue (BPB) dye should turn green by the end of the HCl treatment.*

* U.S. Patent No. 5,112,459.

† Available from Stratagene: Duralon-UV™ membranes (Catalog #420100–420105), Duralose-UV™ membranes (Catalog #420111–420115), and Nitrocellulose Membranes (Catalog #420106–420108).

3. Denature the DNA as follows:

Pour off the HCl and add a 0.5 N NaOH and 1.5 M NaCl denaturation solution. Add enough solution to cover the gel.

Vertical Gels (1.5- or 3-mm spacer)

Treat the gels for 5–15 minutes with gentle shaking.

Horizontal Gels (5- or 10-mm spacer)

Treat the gels for 30 minutes to 1 hour with gentle shaking.

4. Neutralize the denaturing solution as follows:

Pour off the denaturation solution and add a 1 M Tris-HCl (pH 7.5) and 1.5 M NaCl neutralization solution. Add enough solution to cover the gel.

Vertical Gels (1.5- or 3-mm spacer)

Treat the gels for 5–15 minutes with gentle shaking.

Horizontal Gels (5- or 10-mm spacer)

Treat the gels for between 30 minutes and 1 hour with gentle shaking.

**Pretreating the Gels Prior to Northern Transfer
(Formaldehyde Gels)**

1. To allow a more complete transfer for northern blots, it is important to either run a vertical gel or a thin horizontal gel.
2. Following electrophoresis, stain the gel in 5 µg/ml of EtBr in diethylpyrocarbonate (DEPC)-treated deionized water. Destain the gel in DEPC-treated water and photograph it.
3. If the blot will be probed for transcripts ~2 kb or smaller, rinse the gel in two changes of transfer buffer [10–20× SSC buffer (see *Preparation of Reagents*)] to remove the formaldehyde. Proceed to *Assembling the PosiBlot® 30-30 Pressure Blotter*.
4. If the blot will be probed for transcripts larger than ~2 kb, or if the gel is >1% agarose, perform the following pretreatments:
 - a. Soak the gel in 0.05 N NaOH–0.15 M NaCl for 20–30 minutes.
 - b. Neutralize the gel in 0.1 M Tris-HCl (pH 7.5)–0.15 M NaCl for 30 minutes.

Note *Pretreatment times vary depending on the type and thickness of the gel. The following pretreatment times are optimized for horizontal 5-mm-thick formaldehyde RNA gels. Reduce the pretreatment times for thinner vertical gels.*

Preparing the Sponge and the Mask

1. The cellulose sponge (see Figure 1) serves as a buffer reservoir and is shipped desiccated and compressed. To prepare the sponge for use, trim if necessary (before soaking) to a final size of 1.5–2 cm larger than the gel on all four sides. This will keep the required volume of the transfer buffer to a minimum. Soak the sponge in a tray of water for about 5 minutes. Once the sponge is fully rehydrated, wring out the water before saturating with buffer (see step 7 in *Assembling the PosiBlot® 30-30 Pressure Blotter*). This rehydration procedure does not have to be repeated.
2. Carefully cut out a rectangular window in the plastic mask (see Figure 1) that is slightly smaller than the area of the gel that will be subjected to the pressure gradient. This window should be smaller than the rectangle by at least 0.3 cm on all four sides. When cutting the window, make a smooth, continuous cut. Round the corners and avoid "rough edges." The windowed mask can be reused for blotting gels with similar dimensions.

Preparing the Membranes

Note *Always handle the membranes with gloved hands.*

1. Cut the membrane and two pieces of Whatman® 3MM paper 1–2 cm larger than the window in the mask.
2. Prewet the membrane by first soaking it briefly in distilled water (dH₂O) and then in transfer buffer for 5 minutes.

Use 10× SSC buffer, 10× SSPE buffer (See *Preparation of Reagents*), or 25 mM sodium phosphate (pH 6.5) as the transfer buffer for nylon membranes. Use 20× SSC buffer for nitrocellulose or Duralose-UV membranes.

Depending on your application, any of Stratagene's high-quality hybridization membranes may be used.

ASSEMBLING THE POSIBLOT® 30-30 PRESSURE BLOTTER

1. Place the porous membrane support pad with the smooth side down on the supports in the buffer collection base (see Figure 1).
2. Quickly wet one piece of Whatman 3MM paper in the transfer buffer and place the paper on the porous membrane support pad under the window of the mask. Put the mask down and to note the location of the window. Smooth out any air bubbles with gloved fingers or by rolling a pipet lightly across the Whatman 3MM paper.
3. Wearing gloves, place the membrane soaked in the transfer buffer on top of the Whatman 3MM paper (see Figure 1). Smooth out the membrane so that no wrinkles or air bubbles are present.
4. Place the mask over the membrane so that the back edge is lined up at the bottom of the hinge and is centered left and right over the outer edges of the blotter.

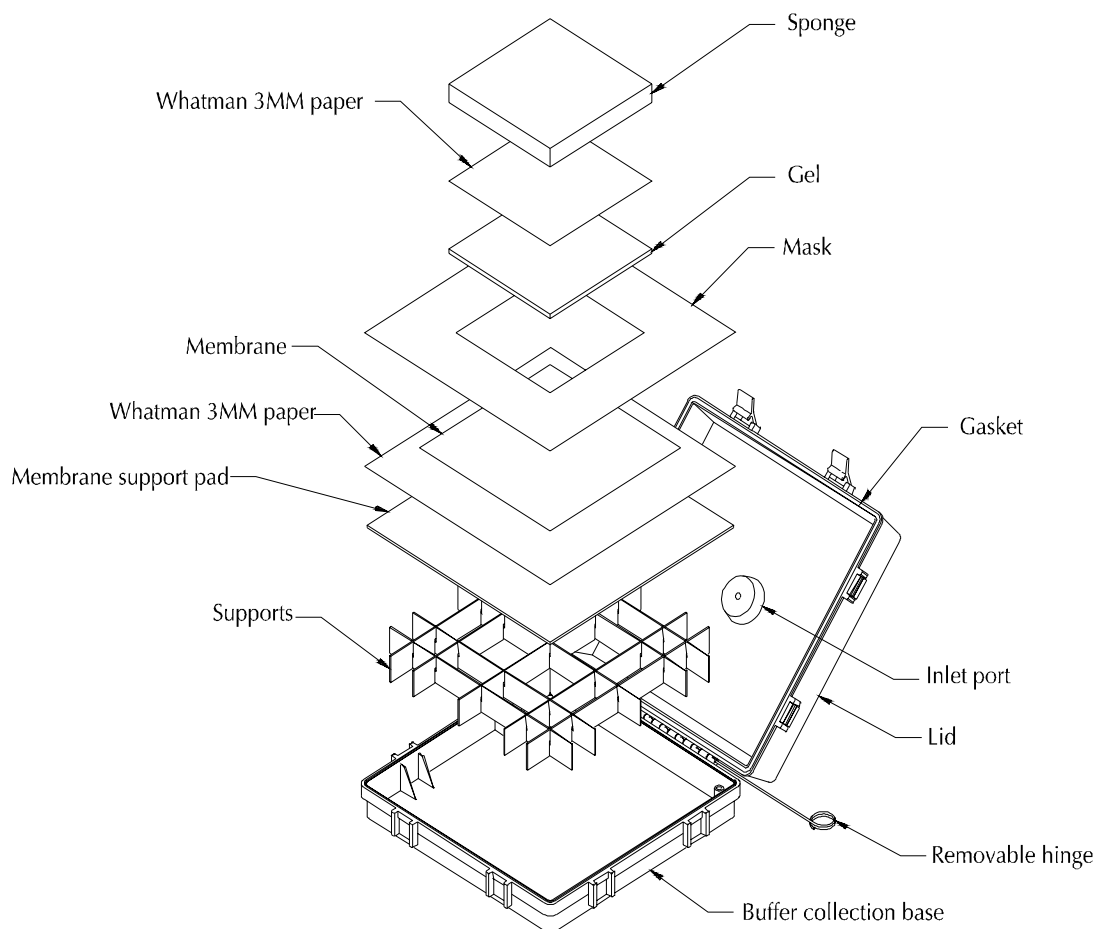


FIGURE 1 Diagram of the PosiBlot® 30-30 pressure blotter.

5. Place the treated gel over the mask so that the upper edge of the rectangular window lines up **below** the row of wells. The other three edges should overlap the gel. If you wish to place the gel wells within the windowed area, the wells should be filled with molten agarose prior to pressure blotting to avoid uneven fluid flow. Gently push out trapped air bubbles under the gel.
6. Quickly wet the other precut Whatman 3MM paper in the transfer buffer and lay the paper on the gel (see Figure 1). Push out any trapped air bubbles.
7. Place the sponge in a tray filled with transfer buffer; the amount of transfer buffer will vary with the size of the sponge. For example, for a small gel sponge (~150 cm²), use 500–600 ml of transfer buffer or, for a large gel sponge (~900 cm²), use 1400–1600 ml of transfer buffer. Saturate by squeezing and releasing, allowing buffer absorption. Raise the sponge out of the fluid and hold it **horizontally** until excess buffer drips instead of streams from the sponge. The sponge **must** be held horizontally at this step to prevent a poor transfer as a result of excessive buffer drainage. Gently lay the soaked sponge over the gel assembly. To ensure complete saturation of the sponge, slowly pour transfer buffer over it until the buffer begins to seep out of the bottom edges.
8. When closing the lid, hold both the side latches that are closest to the hinge up, so that they do not interfere with closing the lid. Place one hand on top of the lid and press the lid down. At the same time, fasten the latches.
9. Turn on the Pressure Control Station. Adjust the pressure to **75 mm Hg** while holding a thumb or hand over the end of the connector hose.
10. Attach the connector hose from the Pressure Control Station to the blotter inlet port. It will take 1–2 minutes for the pressure to rise and equilibrate at 75 mm Hg. If the pressure equilibrates at 70 mm Hg, complete transfer will still occur. Pressure equilibrating at 20–50 mm Hg means there is a leak in the PosiBlot 30-30 apparatus and complete transfer will not occur (see *Troubleshooting*).

Note *Do not attach the PosiBlot 30-30 pressure blotter to anything other than Stratagene's Pressure Control Station.*

11. Blot 30 minutes for thin gels (<3 mm) and 60 minutes for thicker gels (≥5 mm). During blotting, you should see transfer buffer slowly drip into the buffer collection base. If buffer streams into the buffer collection base, there may be a leak in the set-up. Disassemble the blotter and check the integrity of the seal that is formed between the gel and the edges of the window.

Note *Blotting times vary for different gels and depend on the amount and size of the nucleic acid; the size, thickness, and percentage of the gel; the depth of the gel wells; and the volume of sample loaded on the gel.*

12. After the allotted blotting time, turn off the Pressure Control Station and disconnect the connector hose. Open the lid and remove the sponge. If the gel wells are within the windowed area, use a pencil to pierce the gel at the lanes and mark the position of the wells on the membrane. Then remove the gel. The gel may be stained and destained as per step 1 in *Pretreating the Gels* to check the efficiency of transfer.
13. Remove the membrane from the device and place the membrane on clean Whatman 3MM paper to allow the excess buffer to be absorbed. Once the membrane is free of standing liquid, but is still damp, place the membrane and the Whatman 3MM paper in the Stratalinker® UV crosslinker and crosslink on the "autocrosslink" setting (usually ~30–40 seconds). Alternatively, dry the membrane in a 80°C drying oven for 1–2 hours. If blotting onto a nylon membrane, rinse the blot in distilled water to remove excess salt and any fragments of agarose debris.

TROUBLESHOOTING

Observation	Suggestion(s)
The system is not holding pressure	The Pressure Control Station may be malfunctioning. Disconnect the tube from the PosiBlot 30-30 system and place a finger over the tube opening on the Pressure Control Station. If there is no pressure in the tube, the unit needs technical servicing. If there is pressure in the tube, the problem is not with the Pressure Control Station, check the blotter unit seal
	The blotter may not be sealed. If a gel is assembled on the PosiBlot 30-30 pressure blotter, open the lid and wipe off any salt crystals with a damp cloth or push the gasket back into the gasket groove if it has fallen out (see Installing the Gasket). Carefully lift the sponge and Whatman 3MM paper off the gel checking to see if the gel is centered over the window of the mask; melted and cooled agarose can be used to fill in the wells, cracks in the gel, or prevent leaks around the edges of the gel. Let the agarose solidify before assembling the PosiBlot 30-30 pressure blotter. If the problem continues, check the air intake system
The system is not holding pressure	The air intake system may have a leak. Place an uncut mask inside the blotter unit then turn the Pressure Control Station on. If there is no pressure in the tube, make sure the connector hose is secure over the air intake port. Check the connection by putting soap solution on the sides around the gasket, turn the unit on and check for bubbles. If there is an absence of bubbles, then the unit is clamped, and the gasket is adequate. Check for broken or cracked plastic
	If the system is fine but the method used to overlap the membrane, filter and gel is incorrect, check the blotter by placing an uncut mask inside the unit then turning the Pressure Control Station on. Pressure in the tube indicates the unit is fine
The masks are wrinkled	Replace the masks. Store the masks flat and away from extreme light or heat
Poor transfer of the DNA–RNA	The agarose concentration may be too high. Keep the percentage of gel $\leq 0.8\%$ (w/v) agarose
	Thick gels may not transfer efficiently. Make sure the gel is as thin as possible. Extend the blotting time to at least 1 hour

MAINTENANCE

The PosiBlot 30-30 pressure blotter and Pressure Control Station are durable and long-lasting units; however, care must be taken to ensure proper operation and reliable performance. Failure to maintain the PosiBlot 30-30 pressure blotter and Pressure Control Station in the proper condition may reduce their effectiveness.

- ◆ Do not use the pressure blotter without cutting a window in the mask, unless the unit is not holding pressure as indicated in the Troubleshooting section above.
- ◆ Always be sure the PosiBlot 30-30 pressure blotter and Pressure Control Station are on a flat, stable surface.
- ◆ Do not allow the buffer to evaporate inside the gasket groove, since salt crystals will prevent a proper seal. For ease in washing the PosiBlot 30-30 pressure blotter, separate the lid and the buffer collection base by pulling out the removable hinge when the unit is open. Wash the unit in lukewarm water after use and dry the system with a clean cloth. Do not use acetone, alcohol, or chloroform. To reassemble the PosiBlot 30-30 pressure blotter once the unit is dry, place the buffer collection base on a flat surface and align the lid hinge slots with the buffer collection base hinge slots. Insert the removable hinge all the way through the hinge slots to the other end of the unit.
- ◆ Store the mask outside the unit on a flat surface under a book. If the masks warp, place the masks in a 42°C incubator for ~30 minutes.
- ◆ Rinse the membrane support pad with warm water to eliminate possible contaminants.
- ◆ Rinse the sponge in fresh water after each use. Wring out the sponge and let dry.

Installation of the Gasket

1. Begin at a corner and push the flat surface of the gasket straight down into the groove using the thumb or finger of one hand (see Figure 2). With the other hand, stretch the gasket slightly while pushing the gasket down so that the gasket fits into the groove easily.

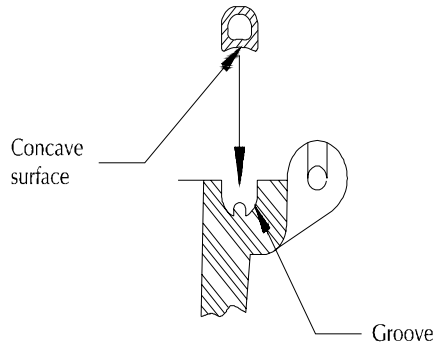


FIGURE 2 Installation of the gasket into the groove.

2. To remove the slack, push the gasket back in the direction of the starting corner to remove any excess tension in the gasket.
3. After the gasket is positioned in the groove, smooth the tension out of the corners and the side sections of the gasket. If the corners are bunched, use your fingers to push the gasket further into the groove and away from the corners. If the corners are lifting up, use your push the gasket with your fingers toward the corner to relieve the tension at the corner.
4. If there is still excess gasket after reaching the starting corner, remove the gasket and begin again, taking care not to overstretch the gasket during installation.

SPECIFICATIONS

PosiBlot® 30-30 pressure blotter	Specifications
Dimensions	
Outer	33 cm W × 33 cm D × 12 cm H
Inner	32 cm × 32 cm (gel support area)
Weight	2.4 kg

Pressure Control Station	Specifications
Dimensions	18 cm W × 19 cm D × 21.5 cm H
Weight	3.2 kg

REPLACEMENT ACCESSORIES

Replacement accessory	Catalog #	Quantity
Masks	#400340	1 set (5/package)
Membrane support pad	#400341	1
Sponges	#400342	1 set (2/package)

PREPARATION OF REAGENTS

20× SSC Buffer (per Liter) 175.3 g of NaCl 88.2 g of sodium citrate 800.0 ml of deionized H ₂ O Adjust to pH 7.0 with a few drops of 10 N NaOH Add deionized H ₂ O to a final volume of 1 liter	20× SSPE (per Liter) 175.3 g of NaCl 27.6 g of NaH ₂ PO ₄ · H ₂ O 7.4 g of EDTA, disodium salt (pH 8.0) 800.0 ml of deionized H ₂ O Adjust pH to 7.4 with NaOH (~6.5 ml of 10 N NaOH) Add deionized H ₂ O to a final volume of 1 liter
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ENDNOTES

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