English

Important user information

Please read this entire manual to fully understand the safe and effective use of this product. Should you have any comments on this manual, we will be pleased to receive them via email at info@immunetics.com or at:

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Boston, MA 02210
USA

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Français

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Español

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Deutsch

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Italiano

Informazioni importanti per l'operatore

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Introduction

Miniblotters are simple, easy to use blotting systems consisting of two clear plastic plates, one smooth and one with channels. The models differ in the number and volume of reaction channels.

The following pages detail the steps for probing a membrane using the Miniblotter.

Select and block membrane

1

Select a membrane of choice (nitrocellulose, Hybond ECL; PVDF, Hybond P; low fluorescent PVDF, Hybond LFP) for the assay.

2

Block membranes with excess non-specific protein.[1]

3

Note: If blocked membranes have been stored in a protein-free solution, re-block them for 1 to 2 minutes in a tray of blocking solution.

[1]See Appendix A, Note 1, Blocking the membrane.

Load membrane

1

Remove the top acrylic plate of the Miniblotter and turn it over.

2

With the antigen-bearing face of the membrane facing the Miniblotter channels, position the membrane so it covers all the channels.[2]

3

Place a new, dry foam cushion (supplied with unit) over the membrane.

4

Set the bottom plate of the unit on the inverted top plate so the alignment pins fall in place.

5

Ensure there is no gap between the top and bottom plates.

[2]See Appendix A, Note 2, Aligning the membrane.

Recommendation: Use a disposable pipette tip connected to a water vacuum aspirator for this purpose.

Important! Take care not to touch the pipette tip anywhere on the unit except in the desired sample hole.

[3]See Appendix A, Note 3, Eliminating bubbles.

[4]See Appendix A, Note 4, Rocker Timing.

Aspirate and introduce antibody solutions

1

Aspirate excess liquid from the channels through the numbered holes.

Note: To avoid drying of the membrane, channels should be loaded within five (5) minutes of aspirating.

2

Pipette antibody solution through the numbered holes.

Press the pipette tip firmly into each hole and inject the liquid rapidly in a single smooth action until the channel is filled. Take care not to introduce bubbles.[3]

model	number of channels	approximate channel volume
MN20SL	20	350 µl
MN28SL	28	50 µl
MN28 Dual	2x28	50 µl
MN16	2x16	110 µl
MN18	18	110-340 µl
MN26	26	110-340 µl
MN25	25	250 µl
MN45	45	110 µl

3

Add buffer to all unused channels that cover the membrane.

Incubate

1

Place the unit on a rocking platform.

Note: For best results, use a slow rocking speed (5 to 6 tilt cycles per minute). A gyratory shaking platform is not effective for this purpose.

2

Incubate the unit on the rocking platform for 30 to 60 minutes at room temperature.[4]

Remove primary antibody solutions and wash the blot

1

Use the washing manifold to remove the antibody solution and wash the blot.

2

Position the 2 identical parts of Washing Manifold in the slots on either side of the top plate and press firmly until the O-rings are seated in the slots.[5]

3

To aspirate all samples simultaneously, first connect the tubing supplied with the unit to both parts of the manifold using luer fitting. Now, connect the open end of the tubing from manifold part to a vacuum source.

Then place the open end of the tubing from the second manifold part into a beaker containing wash buffer.

When you start the vacuum antibody solutions in the channels are removed and the wash buffer is drawn in from the other side. Please refer to your western blotting protocol for wash instructions. For ECL Western blotting we recommend at least 2 quick washes in buffer and two longer (5 min while rocking is enough) before going for the secondary antibodies.

Note: If you have not purchased the manifold, you should rinse channels individually.

This may be accomplished by attaching disposable pipette tips to two lengths of tubing and connecting one to a buffer supply and the other to a vacuum source. Press the two pipette tips into the holes at opposite ends of each channel in turn and flush with buffer for several seconds to wash the blot.

[5]See Appendix A, Note 5, Fitting the O-rings into the slots.

Introduce secondary antibody and incubate

The secondary antibody incubation may be performed either in the unit or in a tray.[6]

To perform the incubation in the Miniblotter unit:

1

Inject the secondary antibody solution into the channels carefully with a single or multi-channel pipette.

2

Incubate the unit on a rocking platform for 30 to 60 minutes at room temperature.

3

Aspirate secondary antibody solution using vacuum source or pipette as described for aspiration of primary antibody solution in the previous page and rinse the blot for a few seconds. Rinsing the blot prevents cross contamination of secondary antibodies across channels when removing the blot from the unit for further processing.

[6]See Appendix A, Note 6, Secondary antibody incubation.

Remove and develop the blot

1. Unscrew the Miniblotter and separate the top and bottom plates.
2. Discard the used sealing pad and wash the membrane in a tray with 3 to 5 changes of buffer for a total of 10 to 15 minutes.
3. Develop the blot by following instructions on your ECL Western blotting system (See also Appendix B).

Clean the Miniblotter

Clean the unit thoroughly after each use by performing the following:

1. Rinse the Miniblotter unit and washing manifold under distilled water.
2. For best results, use Miniclense™. Thoroughly rinse off Miniclense™ before drying.
3. Immerse the Miniblotter unit and washing manifold in the cleaning solution and brush gently with a soft brush taking care not to scratch the interior surfaces.
4. Rinse the Miniblotter unit and washing manifold thoroughly with tap water, followed by distilled water.

   To easily rinse the small numbered holes, assemble the unit and manifold units without a membrane and sealing pad, and flush with distilled water.

Note: Do not autoclave the Miniblotter unit or washing manifold.

Do not expose the Miniblotter unit to alcohol or other organic solvents.

Troubleshooting

problem	possible causes	recommendations
Leaking – antibody solution has moved to adjacent channels.	Dry areas of the membrane act as wicks for fluid from adjacent areas.	Always pre-wet the membrane before mounting in the unit. Fill every channel, covering the membrane with buffer.
	Membrane did not cover the full length of the channel.	Ensure that membrane is cut to proper size.
	Fluid in overloaded channels spilled out when unit was rocked causing contamination of adjacent channels.	Do not overfill channels.
	Re-used foam cushions did not seal properly.	Do not re-use sealing pads as they compress during use.
	Tray incubation.	Consider incubating your blot with secondary antibody in the Miniblotter rather than tray incubation. (See Appendix A: Note 6.)
	Membrane is stripped of blocking protein.	Please take care not to strip the blot of blocking protein while washing it before addition of primary antibodies.
	Unwashed channels contained antibodies that contaminated the experiment.	Clean the unit after every use. For cleaning tips, see the Clean the Miniblotter section, on page 6.
Low Sensitivity	When low affinity or low titer antibodies are used at high dilution (1:100,000 or greater), some decrease in signal intensity may be observed. Antibodies may become depleted in the small volume of the Miniblotter channel.	Increase the antibody concentration 2–5 fold. Increase the antibody volume solution per channel. To do this, insert standard 200 µl plastic pipette tips into both entry ports for each channel. These will serve as reservoirs for sample volumes larger than the volume of the channel itself, while incubating on the rocker. Inject the antibody sample using a tip that then remains in the entry port and becomes such a reservoir. Perform secondary antibody incubations in a tray. (See Appendix A, Note 6.)

Troubleshooting

problem	possible causes	recommendations
Bubbles (***Note:*** Small bubbles generally move when the unit is rocked and do not usually affect results.)	Solutions were too cold. Dissolved gas emerges from the solution as the temperature rises.	Bring solutions to room temperature before loading samples.
	Channels contained residual drops of liquid before samples were loaded.	Tilt the unit and aspirate liquid from the lower end of each channel with a plastic pipette tip attached to a strong vacuum.
	Improper pipetting technique was used.	Seat the pipette tip firmly in the numbered hole and dispense the sample with a single smooth action.
	Foam cushions were moist before mounting.	Ensure that sealing pads are dry before mounting them on the Miniblotter.
	Poor quality or poorly maintained pipette introduced bubbles into the channels.	Try injecting samples with a different pipette or brand of tip.
	Bubbles formed over time.	Place a layer of plastic wrap between membrane and sealing pad.
Manifold is difficult to insert.	O-rings may need lubrication or replacement.	To lubricate: • Remove O-rings carefully with the tip of a flat weighing spatula. • Apply silicone grease lightly. • Reinstall.

Appendix A: Technical notes

Note 1: Blocking the membrane

Membranes should always be blocked *before* mounting in the Miniblotter. Please follow instructions for blocking provided in the ECL Western blotting system.

For detection with western blotting systems use either 5% non-fat dry milk or 5% BSA as blocking agent (See Appendix B).

Blocking with Tween-20 or other detergents alone is *not* recommended as it may cause slow lateral diffusion of proteins through the nitrocellulose membrane. We recommend performing the initial blocking step in a solution containing protein (such as 5% BSA) without detergent. Avoid extensive washes with solutions containing no protein prior to mounting the membrane in the Miniblotter.

In many cases, satisfactory blocking may be attained by incubation with PBS/10% newborn calf serum/0.1% Tween-20 for a minimum of one hour at room temperature. However, blocking agents differ in their efficacy depending upon circumstances. For blots of whole cell lysates, blocking with 50 to 100% serum can be very effective in reducing non-specific binding.

Note 2: Aligning the membrane

For Western blotting experiments it is important that lanes and protein bands on the membrane align with the channels of the Miniblotter unit. This can be accomplished in 2 steps:

Step 1.

Use specially designed Miniblotter combs at the time of electrophoresis. This ensures that the lanes on the gel and, subsequently, the lanes and protein bands on the blot, are aligned with the channels on the Miniblotter.

Combs with well spacings that match the lane spacings of the Miniblotter units are shown below. Please note that the number of wells may not always match the number of lanes in a 1:1 ratio.

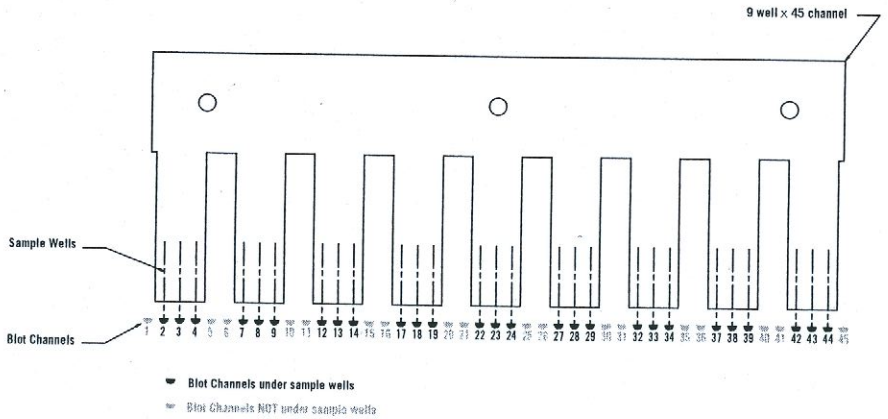

Fig A. This example of a 9-well Teflon comb aligns with the 45 channels of the MN45 and can be used to probe 9 samples with up to three probes per sample.

Note: Comb sizes and patterns vary from manufacturer to manufacturer. Make sure the comb pattern you choose matches up with the Miniblotter channels before proceeding with your assay

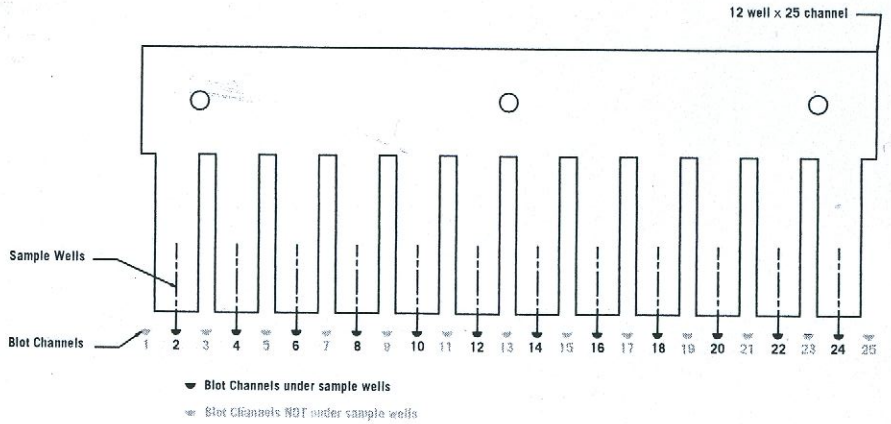

Fig B. This example of a 12-well Teflon comb aligns with the 25 channels of the MN25 and can be used to probe 12 samples with one probe per sample.

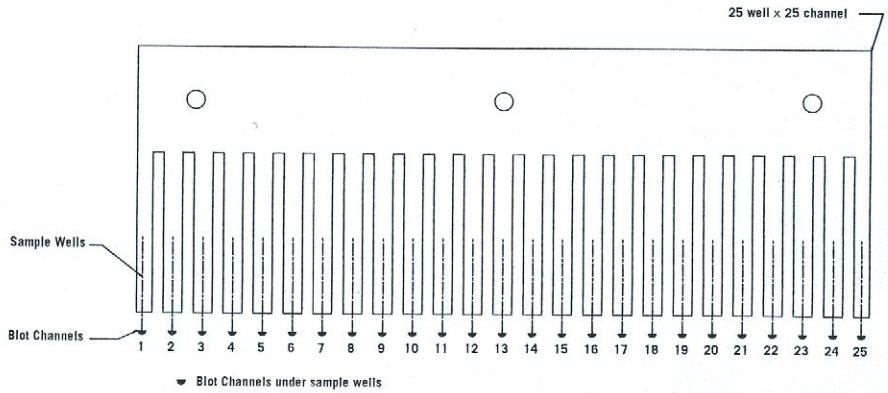

Fig C. This example of a 25-well Teflon comb also aligns with the 25 channels of the MN25 and can be used to probe 25 samples with one probe per sample.

Step 2.

After the gels are blotted, the bands on the membrane are not visible. Adding a non-reactive dye will make it easy to align the membrane. This can be done in one of two ways:

A. For larger protein loads (>250 ng) – Ponceau S can be used to stain the proteins reversibly on the membrane following electrophoretic transfer.

Rinse the membrane briefly in water and stain in 0.2% Ponceau S for 1 to 2 minutes, then destain in several changes of distilled water until red bands appear against a white background. Since the stain is lost during the subsequent blocking step, protein bands should be marked at this stage with pinholes or with a pointed pencil. The blot may also be photocopied.

Ponceau S stained blots can be stored for months at 4 °C, kept moist between sheets of parafilm or buffer to saturated filter paper. Blots can also be frozen in a plastic pouch.

Ponceau S is supplied as a powder that should be dissolved in distilled water to make a working solution. The solution can be reused for several weeks to months depending upon the number of membranes stained.

B. When protein loads are lower than 250 ng (>250 ng), we recommend to use Deep Purple Total Protein Stain for reversible staining of the proteins on the membrane.

After staining the membrane with Deep Purple the protein bands can be visualized using a UV transilluminator (blacklight blue 365 nm wavelength emission is recommended) and marked with pinholes or with a pencil. Deep Purple staining of the membrane is compatible with Fluorescent as well as chemiluminescent Western blotting detection.

If you do not have a UV transilluminator compatible with Deep Purple or for some other reason can not use Deep Purple, methyl green or Pyronin Y may be used to permanently mark the top and bottom of the gel for subsequent alignment with the Miniblotter channels.

When loading the gel, add approximately 5 µl of methyl green (0.1% solution in 50% glycerol) or Pyronin Y (0.05% solution in 50% glycerol) to the desired lanes. These dyes migrate just ahead of the bromophenol blue dye front in the gel, and will transfer permanently to the membrane. A second aliquot of the dye added to the gel near the end of the electrophoretic run will enter the resolving gel in 5 to 10 minutes and serve to mark the top of the gel. Just bear in mind that these dyes are compatible with fluorescent Western blotting detection if these bands and the bromophenol blue is removed before the detection. If left on the membrane they will result in unspecific signals in fluorescent Western blotting detection.

Note 3. Eliminating bubbles

Small bubbles in the channels generally do not affect the end reaction, and should move back and forth over the surface of the membrane when the Miniblotter is rocked. To eliminate large bubbles, withdraw the solution from the channel and re-inject. For further information, refer to the Troubleshooting section.

Note 4. Rocker Timing

The angle and timing of the rocker can vary by experimental design. These criteria should be optimized for each application.

Note 5. Fitting the O-rings into the slots

If your washing manifold units do not fit into the slots easily, apply a small amount of silicone grease around the O-rings. Place the manifold unit loosely in the slot and press down on the side of the manifold towards you. Then press on the side away from you to seat the manifold firmly in the slot.

Note 6. Secondary antibody incubations

Note: Exercise caution when performing tray incubations because low affinity monoclonal antibodies can dissociate from their respective antigens over time. Incubation in a tray permits such antibodies to diffuse through the incubation solution and rebind elsewhere on the blot. Such *streaking* is detectable as staining between sample lanes or in negative control lanes at the position of one or more strongly reactive bands.

Please follow instructions provided with your Western blotting system. When secondary antibody is used in the Miniblotter at a high dilution (1:100,000 or greater), some decrease in signal intensity may be observed. This may be due to depletion of antibodies in the small volume of the channel. The problem can generally be corrected by 1) increasing the antibody concentration 2–5 times, 2) increasing the antibody volume, or 3) removing the membrane from the unit and performing the secondary antibody incubation in a tray, 4) performing the secondary antibody incubations on the Miniblotter while rocking the unit on a rocker.

Appendix B: Detection using Western blotting systems

There are several Western blotting systems available.

The following "general" protocol is suggested:

❶

After electrophoresis and transfer of separated proteins to nitrocellulose or PVDF (low fluorescent Hybond™ LFP for ECL Plex) membrane, block unspecific sites with suitable blocking solution.

❷

Blocking is followed by two quick washes in PBS/0.1%Tween (PBS-T).

❸

Immerse and incubate the membrane in antigen specific primary antibody of optimized concentration.

❹

Membrane is rinsed in two quick washes of PBS-T followed by 2 longer washes.

❺

Incubate the membrane in optimized concentration of the conjugated secondary antibody.

6

Briefly rinse the membrane with two changes of wash buffer followed by 4 longer washes in PBS.

7

Briefly wash the membrane three times in PBS before detection of the fluorescent dyes with laser scanner.

8

Drain off excess detection reagent and wrap the membrane in SaranWrap before exposure to X-ray film.

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Appendix C: Accessories, spare parts and related products

product	qty.	order no.
Accessories and spare parts		
Plastic Sealing Pads	10	PC1, PC2
Plastic Sealing Pads	100	PC100, PC200
Washing Manifold Kit	1	F1, F2
O-ring Seals	2	OR1, OR2
Manifold Luer Connectors	4	LC002
Derlin Screw	1	SR2
Miniclense™	1	CC-B006-060
Accessory Kit	1	MAP-01, MAP-02
Silicone Lubricant	1	MSL-01
Tubing Adaptor Assembly	1	MTAA-01