

Tips and tricks of the trade

Dot Blot in the Box

One of the most frustrating moments a researcher may face comes when an established method fails, regardless how hard he tries to get it running. Failing methods, however, are also a great motivation to develop new techniques.

Lab Hint

During my diploma thesis, which was supervised by Thomas Arendt and Wolfgang Härtig at the Paul Flechsig Institute for Brain Research in Leipzig, Germany, I had to quantify brain proteins such as tau and doublecortin. Since this is usually done by Western blotting, I checked all available Western protocols and worked hard to get them running. However, as much as I tried, some lanes didn't want to develop and others remained unstained after stripping the membrane – even when I used the same antibody.

To fix these problems, I initially tested different conditions for antibody incubation. I obtained a better staining by agitating the blotting membrane harder in the antibody solution. Unfortunately, this led to very high antibody consumption, so I changed my strategy and started to incubate blotting membrane confetti in 96-well-plates. I let an acryamide gel run for some millimetres, blotted the proteins onto a PVDF-membrane and cut the membrane into single “lanes” with a scalpel and transferred the confetti into a 96-well-plate. I actually achieved a stable staining when I performed all of the necessary incubation steps within these plates.

However, two problems still remained. Firstly, the lanes were too wide to fit into the wells in one piece. Secondly, having 34 samples, to allow at least a 4-fold determination, made producing the membrane confetti this way too laborious. A dot blot device was not available in the lab and an enzyme-linked immunosorbent assay (ELISA) failed due to the lower antigen binding capacity of ELISA plates compared to PVDF membranes. Establishing ELISA plates with a capturing antibody was too time-consuming and buying a custom-made ELISA for several thousand euros was impossible.

So what could be done? After some further experiments, I developed a cheap and rather easy alternative dot blot method to transfer protein samples to a PVDF membrane receiving high quality spots.

Here is how the alternative dot blot works (you may find a detailed descrip-

tion in our recently published paper: M. Lange, T. Arendt & W. Härtig, *Anal. Biochem.* 398:129-131).

Wet a PVDF membrane of appropriate size in methanol and equilibrate it in blot buffer, according to the protocol described by Bjerrum and Schafer-Nielsen (*Analyti-*

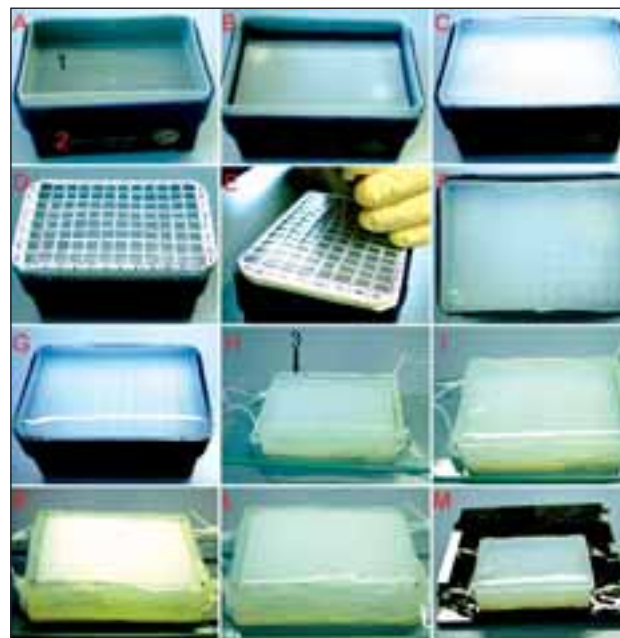


Fig. 1, showing step-by-step instructional pictures of the alternative dot blot method.

cal Electrophoresis, M. J. Dunn, ed., 1986, p. 315; Verlag Chemie, Weinheim). Lay the membrane in a flat plastic box (e.g. a recycled lid of a tip box), cast it in with approximately 50 ml agarose in blot buffer (Fig. 1 B) and cool the plastic box on ice for some minutes (you may use the tip box as an ice container). The first agarose gel solidifies and fixes the membrane near the bottom of the box (part 1 in Fig. 1 A) before the box is completely filled with agarose gel (Fig. 1 C). After hardening the gel in the fridge for half an hour, place a grid onto the gel as shown in Fig. 1 D.

In the next step, directly inject the protein samples (solubilised in Laemmli's sample buffer) into the gel (2 μ l per position) using a micro syringe (Fig 1 E). Remove the grid after injecting all samples (Fig 1 F) and cover the top of the gel with a thin layer of

agarose to prevent any sample leakage (Fig 1 G). Remove the gel from the casting box and transfer it into a electrophoresis frame (a box without bottom, e.g., prepared from the lid of an old tip box, see Fig 1 H). Fix the gel block to the frame using agarose gel (Fig 1 I to L) and knot the frame onto a blotting

cassette suitable for Western tank blotting, before transferring the proteins over night at 100 mA.

At this point, you have mastered the most delicate parts of the protocol, the rest is routine. Free the membrane from the gel, wash it in Tris-buffered saline containing 0.5 g/l Triton X-100 (TBS-T), stain it with Ponceau-S and cut out the stained dots (5.5 mm diameter) with a conventional office puncher. Transfer the obtained confetti into the water-filled wells of a standard black 96-well-plate (6.0 mm diameter) and add 0.1 % sodium hydroxide to remove Ponceau-S (this will take approximately five minutes, depending on the staining intensity). Wash the membrane extensively with TBS-T to recover a physiologic pH value and apply it to a standard immunostaining procedure, including specific primary antibodies and conjugates of secondary antibodies and horseradish peroxidase. Finally, you may detect the proteins by a luminometer using para-hydroxycoumaric acid-enhanced luminol chemiluminescence. If you want to check another antibody, you may reprobe the confetti after stripping it with any established stripping protocol.

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Do you have any useful tips?

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