Anti-HA antibody Western Blotting

Marc Diamond's method
SJF 4/00

1) Run gel (in 1:10 running/transfer buffer (10x) and H2O for a total of 1litre) at 150 volts until leading bromophenol blue band is nearing the bottom edge of the glass plates or according to the expected migration of the protein of interest.

2) Transfer proteins in acrylamide gel to nylon membrane.

3) Block overnight @4ºC in PBS (or TBS, it doesn't matter) with 5% non-fat dry milk. I dilute 2.5 mg in 50 ml.

4) Wash off excess milk solution with PBS x3 (important if you are re-using your antibody).

5) Incubate in primary antibody solution: I use PBS with 0.1% TWEEN-20 plus 5% BSA as the basic solution, but you can use TBS too. I dilute the HA antibody 1:5000, just to be sure you will see a signal. I haven't EVER seen appreciable background. I've also used it at 1:10K without any appreciable loss of signal. I usually go 30–90 minutes at RT (you can save the 1ab solution and use for a month or so).

6) Wash with 12–15 quick washes with PBS/Tween (I literally pour it in, shake it around for 5–10 seconds, and pour it off: this method was worked out in the Yamamoto lab eons ago).

7) Incubate with secondary antibody (anti-mouse) in 5% BSA/PBS/Tween as you would ordinarily. I usually go for 2 hours @ room temp.

8) 12–15 quick washes again (in 1XPBS).

9) Assay for chemiluminescence or whatever you want. The normal procedure is to submerge blot in 20 ml of ECL reagent for 2 minutes. Then wrap blot in saran wrap and wipe away excess ECL reagent. Place blot in film cartridge and bring to dark room. Expose film for ~2 mins.

Notes on the primary antibody:

Source - Covance/Babco

Description: Crude ascites fluid monoclonal anti-HA antibody (CATLN#135032002, 11mms-101R, 0.5 ml)

Handling: Aliquoted into 10ul portions in –20ºC

Internal notes: labeled anti-HA (Marc's 1:5000)