Western Blot on ATPROTEINCHIP 1

Materials:

SuperBlock Blocking Buffer
Anti-cMyc antibody
Cy5 anti-mouse IgG antibody
1xTBST
Aluminum foil

Equipment:

Blotting container
Shaker

1. Remove protein slide from -80^{o}C, put into the incubation tray/blotting container with the side with protein spots facing up, and equilibrate at 4^{o}C for at least 15 minutes.
2. Block the slide in SuperBlock Blocking Buffer in TBS for 1 hour at room temperature, while shaking gently (~50 rpm). Be sure that the spots on the slides are facing up during incubation.
3. Carefully remove the blocking buffer and add the primary antibody anti-cMyc (9E10), 1:1000 in blocking buffer, add to blotting container, but avoid pouring directly onto the slide. Incubate for one hour in room temperature, with gentle shaking (50 rpm). Remove primary antibody solution by decanting the solution from the box. Use a 1mL pipette to remove any residual solution that remains. Take care not to disturb the slides.
4. Wash the slide. Carefully add ~10 mL 1X Tris-Buffered Saline with 0.1% Tween (TBST) into the chamber, avoid disturbing the slide surface, incubate for 5 minutes at room temperature, with gentle shaking (50rpm). Decant off TBST and repeat wash two more times.
5. Carefully add the secondary antibody in blocking buffer (1:4000 Cy5 conjugated anti-mouse IgG) into the tray with the slide, incubate for 1 hour at room temperature, on shaker with constant shaking (50rpm). Seal the boxes with foil to avoid light exposure.
6. Wash the slide. Repeat step 4. Seal the boxes with foil to avoid light exposure during incubation.
7. Use forceps to place the slides inside 50 mL falcon tubes sealed with foil. Spin dry at 1,000 rpm. Put the slides inside a slide box to dry for 10 min. Scan the slide using the Affymetrix 428 or GenePix 4100A scanner, using 635 nm channel.
8. Save scanned images in multilayer TIFF format, for future data analysis. A grid file is provided with information for protein spots location and identity.

This protocol was tested at the ABRC, enabled by OPBC funding #2011-003 to Jelena Brkljacic

ATPROTEINCHIP 1 probing

Materials:

- Probing buffer - PBS (137 mM NaCl; 2.7 mM KCl; 10 mM Na_2HPO_4; 1.76 mM KH_2PO4)
- Washing buffer (Probing buffer containing 0.1% Tween 20 and 1% Glycerol)
- Labeled, purified protein (e.g. using Alexa Fluor® 647 Protein Labeling Kit, Molecular Probes)

Equipment:

- Fluorescent slide scanner

1. Apply 120 µl probing buffer, containing 5-50 µg/ml of purified labeled protein, to each slide and cover with HybriSlip™ cover (Grace Bio-Labs). Incubate at room temperature in a humid chamber for 1 hour.
2. Wash 3 times for 5 minutes with washing buffer. Spin dry.
3. Scan slides using Affymetrix 428 or GenePix scanner.

This protocol was tested at the ABRC, enabled by OPBC funding #2011-003 to Jelena Brkljacic