pIMAGO-based detection on dot-blot

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1) Prepare the **blocking buffer**: 4mL of 1% BSA in 1xTBST containing 15uL PAMAM G4 dendrimer (keep all ratios the same if using larger volume to cover the membrane).

2) Cut PVDF membrane into smaller pieces (e.g. 3x2 in.). Do not use nitrocellulose.

3) Dip the membrane briefly in methanol, drain excess for 2-3 secs, and lay the membrane down onto cellophane wrap.

4) Spot you proteins before the membrane dries out (e.g. can spot 0.5-1ul of each protein; should be a total of 100-200ng of each protein spotted).

5) Let the membrane air-dry a bit so the spots dry, but do not let the membrane dry out completely because it will be difficult to block.

6) Block the membrane for 1hr with the **blocking buffer** (or overnight at 4 degrees). Let membrane with blocking buffer shake or rotate - the whole membrane needs to be covered.

7) Dump out the buffer and rinse the membrane once with the **pIMAGO buffer** (currently, 500mM glycolic acid, 1% TFA)**.**

8) To prepare pIMAGO solution, in a separate tube add 5uL of **pIMAGO reagent** to the amount of **pIMAGO buffer** (currently, 500mM glycolic acid, 1% TFA) necessary to cover the membrane (usually 4 mL for dot-blot). Vortex and add to the membrane, incubate 1hr.

9) Dump our the solution and wash the membrane 4 times with the **pIMAGO buffer** (5 min each time); and 2 times with **1x TBST** (5 min each time).

10) In a separate tube, prepare 1:5,000 mixture of **Avidin-HRP** in the buffer of **1% BSA/1x TBST** (usually 4 mL). Vortex and add to the membrane, incubate 45 min.

Alternatively, streptavidin-IRDye can be used instead of avidin-HRP, to enable fluorescence-based detection.

1) Dump out the solution and wash the membrane 4 times with **1x TBST** (5 min each wash).

7) Detect the signal as usual using HRP chemiluminescent substrate. (Do not expose the film for more than 2-3 min to avoid high background).

   To detect the signal: a) in a separate tube, mix ECL reagent A with ECL reagent B at 1:1 ratio (usually 1mL of each is enough). b) Take out the membrane and place it face-up on a cellophane wrap. c) Pipet the ECL mixture onto the membrane to completely cover it, let stand 1minute. d) Drain the excess solution from membrane and put the membrane onto a clear plastic wrap, remove any bubbles between membrane and plastic. e) In the dark room, put the plastic-covered membrane into a cassette, put a film on top, close the cassette and expose 0.5-2min. f) Dip the film in the Developing solution for 5seconds, take out, and when the signal is satisfactory, dip the film into Fixing solution for 30 seconds. g) Rince with water.

   Important - do not expose the film to light (make sure the lights are turned off when exposing the membrane.