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Protocol

pIMAGO-biotin Phosphoprotein Detection on Western Blot

Components provided:

Phosphorylated protein (β-casein) in LDS sample buffer as a control (load 10µL in a well; store at 4ºC);

10x Blocking buffer (need to dilute to 1x with DI water; store 10x and 1x buffers at 4ºC);

5x pIMAGO buffer (need to dilute to 1x with DI water; store at 4 to 25ºC);

10x Washing buffer (need to dilute to 1x with DI water; store at 4 to 25ºC);

5x IAA solution (store at 4ºC);

pIMAGO reagent (store at 4ºC);

avidin-HRP, if ECL-based detection (store at 4ºC);

avidin-Fluor, if fluorescence-based detection (store at 4ºC);

Need to prepare: 1x TBST (Tris-buffered saline with 0.1% Tween 20);

Protocol

1. Before running the gel, boil the samples in SDS/DTT and let them cool down to room temp. Add **5x IAA** solution to a 0.5x-1x final concentration directly to the samples and incubate in the dark for 15 min (this step is optional but can improve detection specificity). Load the samples onto a gel. Load one well with 10 µL of the provided phosphoprotein as a positive control (load as is, no need to boil).

2. Run your samples and transfer onto a membrane (Tris-glycine transfer buffer provides the cleanest results).

If it is desired to do fluorescent-based detection, use a special membrane with low autofluorescence.

Important Note: In many cases, the transfer system itself might contain contaminants, increasing the nonspecific background signal. To reduce this, we strongly recommend including a second piece of membrane before the gel to bind any of these contaminants (suggested set-up: filter-membrane-gel-membrane-filter). Not necessary for nitrocellulose.

3. Block the membrane for 1hr with a **1x Blocking buffer** (e.g. 10 mL for a mini blot; this step can also be carried out overnight at 4ºC).

4. Prepare 1:1,000 mixture of **pIMAGO reagent** in **1x pIMAGO buffer** (e.g. 10 µL pIMAGO in 10 mL pIMAGO buffer for mini gel). Mix and add to the membrane, incubate 1 hour.

5. Wash the membrane 3 times with 10-20mL of **1x Wash buffer** and once with **1x TBST** (5 min each wash).

6. Prepare 1:1,000 mixture of **avidin-HRP or avidin-Fluor** in the **1x Blocking buffer** (e.g. 10 µL avidin reagent in 10 mL of blocking buffer for mini gel). Mix and add to the membrane, incubate 1 hour.

7. Wash the membrane 3 times with **1x TBST** (5 min each wash).

Detect the signal as usual using scanner or HRP chemiluminescence substrate. (Typically, do not need to expose the film for more than 1-2 min to avoid high background; no need to dry the membrane for fluorescence detection).

Note: For nitrocellulose membrane, it is sometimes observed that HRP might go through ECL substrate too fast and no signal is detected. In this case, rinse the membrane with TBST and add more ECL substrate for repeat detection.

Store the kit at 4°C