18419 pIMAGO®-biotin Phosphoprotein Detection Kit, for western blot, HRP-based detection (ECL)

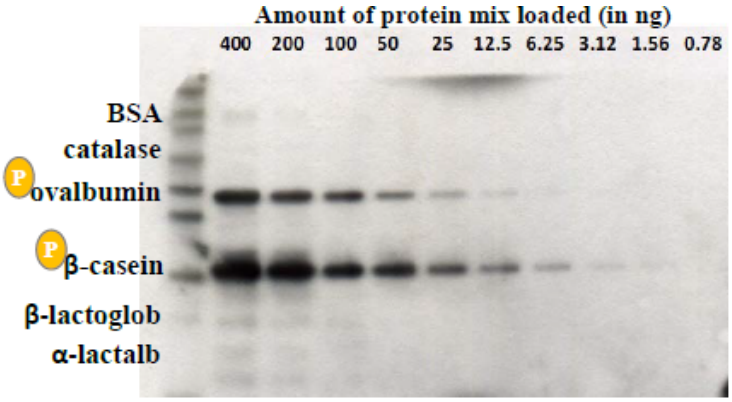
Introduction

pIMAGO® is a universal phosphoprotein detection technology that enables sensitive and specific recognition of phosphorylated molecules. Unlike phospho-antibodies, the binding is not biased by amino acid sequence, and therefore can be used for detection of any phosphorylation event on any protein site. pIMAGO® detection protocol resembles a simple Western Blot procedure and can be easily incorporated by any laboratory.

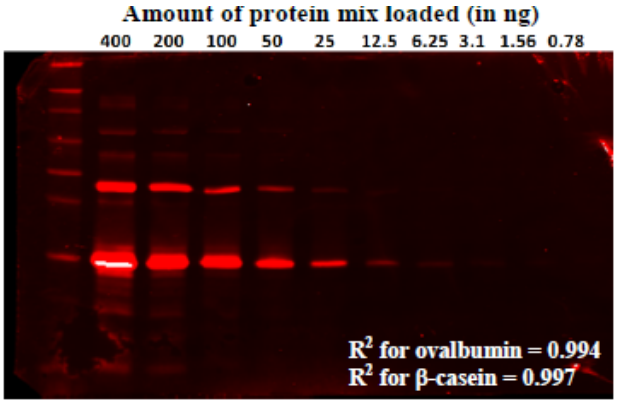
Results

pIMAGO® analysis of 6 protein mixture (2 phosphoproteins) avidin-HRP or avidin-Fluor detection.

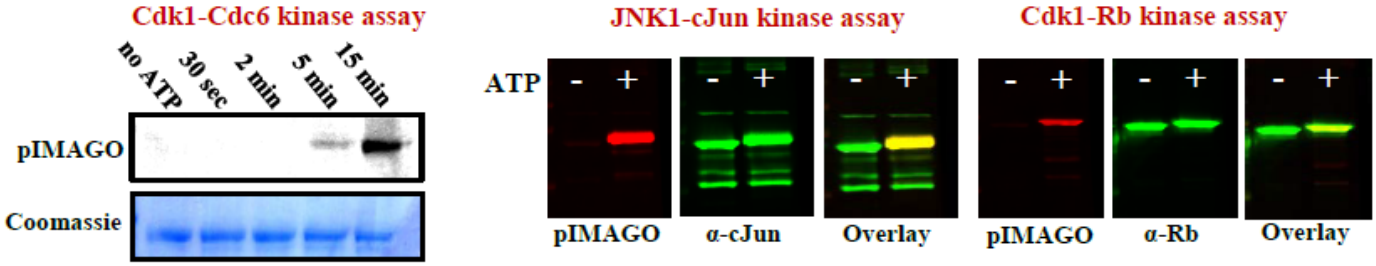
Avidin-HRP detection



Avidin-Fluor680 detection



pIMAGO® analysis of *in vitro* kinase assays using avidin-HRP (left) or avidin-Fluor (right) detection.



Kit components

400µL of pIMAGO® reagent
400µL of avidin-HRP
2mL of 5x IAA
80mL of 10x Blocking buffer
80mL of 5x pIMAGO® buffer
240mL of 10x Washing buffer
400µL of control phosphoprotein

Protocol

- 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).
- 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.

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

Original citation.

Iliuk A., Liu XS., Xue L., Liu X., Tao WA. (2012). Chemical visualization of phosphoproteomes on membrane.
Molecular and Cellular Proteomics. **11**: 629-639.
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Storage : 2-8°C

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.