19379 pIMAGO®-biotin Phosphoprotein Detection Kit, for Microplate Blot, HRP-based detection (400 wells)

Storage Temperature 2-8°C

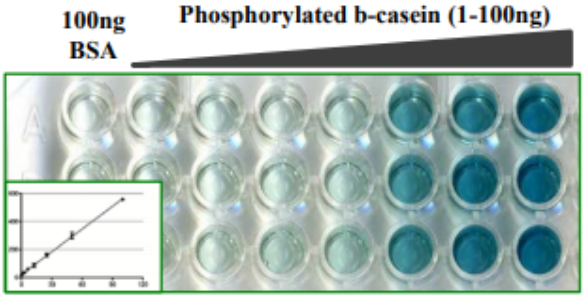
Introduction

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 ELISA procedure and can be easily incorporated by any laboratory. Due to its small size, pIMAGO® can be multiplexed with antibodies for simultaneous detection of phosphorylation and total protein amount.

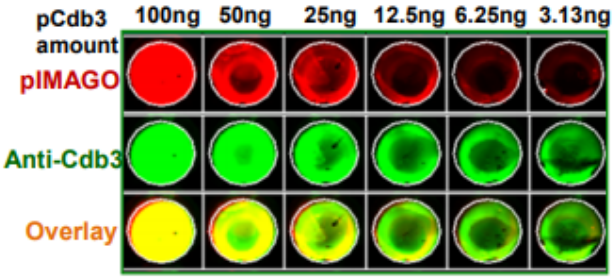
Results

pIMAGO-based detection and quantitation of phosphorylated ☐-casein signal compared to non-phosphoprotein BSA (*left figure*). Multiplexed detection of phospho-Cdb3 using pIMAGO in the 700 channel and anti-Cdb3 antibody in the 800 channel *(right figure*). pIMAGO-based kinase assays of 5 kinases and their substrates, including control, ATP, and ATP + Kinase wells (*bottom figure*).

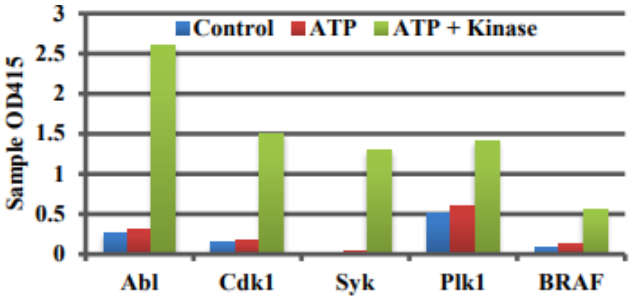
pIMAGO colorimetric detection detection



pIMAGO fluorescence-based assays



pIMAGO detection of *in vitro* kinase assays



Kit components

- 400 µL of pIMAGO reagent
- 400 µL of avidin-HRP
- 40 ml of Binding buffer
- 240 mL of Blocking buffer
- 240 ml of pIMAGO buffer
- 40 ml of Colorimetric substrates
- 60 ml of Stop solution
- 200 µL of control phosphoprotein (☐-casein; mix 5 ☐l with 95 ☐l Binding buffer)
- 10 flat-bottom clear 96-well plates

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

Protocol

1. Binding of samples to the 96-well plate
   Prepare a protein solution of your sample (phosphoprotein or substrate of interest) in **Binding buffer**. A dephosphorylated (*e.g.* alkaline phosphatase-treated or without ATP) form of the sample should also be prepared as a reference. If protein amount is known, use 10 to 500 ng of the protein or mixture of proteins per 100 µl of **Binding buffer** per well. Add 100 µl of the mix into each well on a 96-well plate. Incubate overnight at 4°C at 400-600 rpm to bind the proteins to the plate. As a positive control, in a separate well mix 5 µL of the provided phosphoprotein with 95 µL of Binding buffer.
2. Blocking the wells
   Remove solution from wells, add 150 µl of the **Blocking buffer** into each well and incubate 2-3 minutes. Remove the solution and add 150 µl of the **Blocking buffer** again and incubate for 30 min while shaking at 400-600 rpm at room temperature.
   At this stage, any additional manipulations can be carried out (e.g. kinase/phosphatase assay, inhibitor screening, etc.). Make sure to wash the wells 3x with the **1x TBST after each manipulation.**
3. pIMAGO incubation
   In a clean tube, prepare a 1 to 100 mixture of the **pIMAGO reagent** in the **pIMAGO buffer** (1 µl of the reagent for every 100 µl of buffer). Empty the wells and add 100 µl per well of the prepared pIMAGO mix. Incubate 1 hour at 400-600 rpm at room temperature.
4. Washing the wells
   Empty the wells and add 150 µl of the **pIMAGO buffer** into each well; incubate 2-3 minutes at 400- 600rpm. Remove the buffer and repeat the washing step two more times with the **pIMAGO buffer** for a total of 3 washes. Remove the solution and incubate the wells with 150 µl of the **Blocking buffer** for 15 min at 400-600 rpm at room temperature.
5. Incubation with avidin-HRP
   In a clean tube, prepare 1 to 100 mixture of **avidin-HRP** in the **Blocking buffer** (1 µl of **avidin-HRP** in 100 µl of **Blocking buffer**). Empty the wells and add 100 µl per well of the prepared avidin-HRP in blocking solution. Incubate the plate for 1 hour at 400-600 rpm at room temperature.
6. Washing the wells
   Empty the wells and add 150 µl of **1x TBST** into each well; incubate 2-3 minutes at 400-600 rpm and remove the solution. Repeat the washing step with TBST two more times for a total of three washes. Empty the wells.
7. Signal detection
   For normal and high concentrations of the proteins (majority of in vitro samples), use the provided colorimetry-based detection system. Prepare 9 to 1 mixture of the **Colorimetric Substrates A and B** (Note: *has to be made fresh each timeefore detection*), and add 100 µl to each well. Shake the plate until satisfied with signal – solution will turn green if signal is present (usually 1-2 min), then add 150 µl of the **Stop solution** to stop the HRP-substrate reaction. Read the plate at 415 nm in a plate reader.
   ** Alternatively, any other peroxidase substrate can be used (chromogenic or chemiluminescent). However, for chemiluminescence-based detection, a different plate with non-transparent walls must be used. **

References

"*Phosphorylation assay based on functionalized soluble nanopolymer*". Iliuk, A.; Martinez, J.; Hall, M. C.; Tao, W. A.,
Anal. Chem. **2011**, 83, 2767-2774. PMID: 21395237

Legal Information

pIMAGO® is a registered trademark of Tymora Analytical Operations, LLC

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.