Cova-PIP ELISA Protocol

Summary

Detects protein binding to phosphoinositides in a 96-well microtiter plate
Similar to common ELISA protocols
Estimated time: 3-4 hours, several steps can be extended overnight if needed

Materials and Solutions

Microtiter plate: Cova-PIP plate manufactured at Echelon, pre-blocked and ready to add protein of interest.

Solutions:
TBS: Tris-Buffered Saline, 10 mM Tris, 150 mM NaCl, pH 7.8 to 8.0
TBS+G.S: Add 0.5 mL goat serum (GibcoBRL # 0885) to 49.5 mL TBS to make TBS + 1% Goat Serum
TBS-T: Add 50 µL Tween-20 (J.T.Baker #X251-07) to 100 mL TBS to make TBS + 0.05% Tween-20

Antibodies
Mouse anti-GST (Sigma # G-1160 or equivalent)
Goat anti-mouse IgG-HRP (Sigma # A-9917; Jackson IR Labs # 115-035-146 or equivalent)
Goat anti-mouse IgM-HRP (Sigma # 8786; Jackson IR Labs # 115-035-020; or equivalent)

Peroxidase Substrate & Stop Solutions
3,3',5,5'-Tetramethyl benzidine liquid, TMB (Sigma # T-8665 or similar)
0.5 M Sulfuric Acid (dilute concentrated H_2SO_4 1:35 with water)
CAUTION! *Both TMB and H_2SO_4 are toxic. Wear appropriate protective clothing and use proper technique when preparing and using these chemicals.*

Method

1. **Add protein** of interest (100 µL/well, diluted in TBS+G.S). {Suggested starting concentrations: PH domain protein ~ 1 µg/mL; anti-PIP IgG ~ 0.5 µg/mL; and anti-PIP IgM ~ 2.5 µg/mL}
2. **Incubate:** cover plate to minimize evaporation and incubate for at least one hour at room temperature. We recommend gentle agitation on an orbital plate shaker for this and other incubation steps. Longer incubation times or static incubation overnight at 4 °C are acceptable.
3. **Wash:** discard protein or antibody solution in sink or waste receptacle and wash plate 3-5 times with TBS-T using an automatic plate washer; or wash manually by adding 150 µL/well TBS-T and let sit for 2-5 minutes between each wash. If high background binding is observed, we recommend additional washes for longer periods of times, and perhaps agitating the plate on an orbital plate shaker during the wash steps.
4. **Add secondary antibody** (100 µL/well, diluted in TBS+G.S. according to manufacturer's instructions) and incubate 30 min to 1 hour at room temperature. For Sigma antibodies, Echelon recommends using anti-GST at 1:1,000; Goat anti-mouse IgM-HRP at 1:2,000; and Goat anti-mouse IgG-HRP at 1:3,000 dilutions.
5. **Wash** plate as in step 3.
6. *If necessary,* **add tertiary antibody** (100 µL/well, diluted in TBS+G.S. according to manufacturer's instructions) and gently agitate for 30 min to 1 hour at room temperature.
7. **Wash** plate as in step 3.
8. **Add peroxidase substrate and develop** according to manufactures instructions. We add 100 µL/well TMB, incubate in the dark at room temperature for 3-30 minutes and stop the reaction by adding 50 µL/well of 0.5 M H_2SO_4 when significant blue color develops.
9. **Read** the absorbance with a plate reader at the appropriate wavelength. If using TMB substrate, read at 450 nm after adding stop solution.