



User's Manual

INPP4 Activity ELISA Kit

REF DEIA-XYZ19

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The INPP4 Activity ELISA Kit is a competitive ELISA which measures the activity of INPP4A and INPP4B.

General Description

INPP4A is a magnesium-independent phosphatase involved in the phosphatidylinositol 3-kinase (PI3K) pathway. INPP4A and its isoenzyme INPP4B hydrolyze the 4-phosphate from the lipid second messenger phosphatidylinositol-3,4-bisphosphate (PI(3,4)P₂) to form phosphatidylinositol-3-phosphate (PI(3)P). While little evidence supports its role as a tumor suppressor, INPP4A suppresses glutamate excitotoxicity in the central nervous system and is responsible for the Weeble mouse phenotype. A spontaneous frame shift mutation in the *Inpp4a* gene is present in the Weeble mouse resulting in selective neuronal loss in the cerebellum resulting, severe ataxia and early death. The INPP4 ELISA is a competitive assay in which the signal is inversely proportional to the amount of PI(3)P produced. After the INPP4 reactions are complete, reaction products are added to the PI(3)P-coated microplate for competitive binding to a PI(3)P detector protein. The amount of PI(3)P detector protein bound to the plate is determined through colorimetric detection. The assay displays excellent robustness (Z' value ~ 0.8).

Reagents And Materials Provided

1. Detection Plate, 12 x 8-strip well plate: 4°C, 1 Plate
2. PI(3,4)P₂ Substrate, diC8: -80°C, 100 µg
3. PI(3)P Standard, diC8: -80°C, 3 µg
4. 5x PI(3)P Detection Buffer (5x DB): 4°C, 4 mL
5. INPP4A Enzyme Positive Control: -80°C, 2.5 µg
6. 5x INPP4 Reaction Buffer: RT or 4°C, 1 mL
7. 10x TBS-T Buffer: RT or 4°C, 20 mL
8. PI(3)P Detector: -80°C, 4 x 10 µg/vial
9. Secondary Detector: 4°C, 300 µL
10. TMB Solution: 4°C, 12 mL
11. 1 N H₂SO₄ Stop Solution: RT or 4°C, 10 mL
12. Clear acetate sheet, 1 side adhesive: RT or 4°C, 3 pieces

Materials Required But Not Supplied

1. INPP4 samples for testing
2. Absorbance plate reader capable of reading at 450 nm

Storage

Part 1 at 4°C, Part 2 at -20°C