



## **Inpp5d ELISA Kit (Rat) (OKEH06808) Instructions for Use**

For the quantitative measurement of Inpp5d in serum, plasma, tissue homogenates, cell culture supernatants and other biological fluids.

Lot to lot variations can occur. Refer to the manual provided along with the kit.

This product is intended for research use only.

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## 1. Background

### Principle

Aviva Systems Biology Inpp5d ELISA Kit (Rat) (OKEH06808) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for Inpp5d has been pre-coated onto a 96-well plate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for Inpp5d is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample Inpp5d captured in the well.

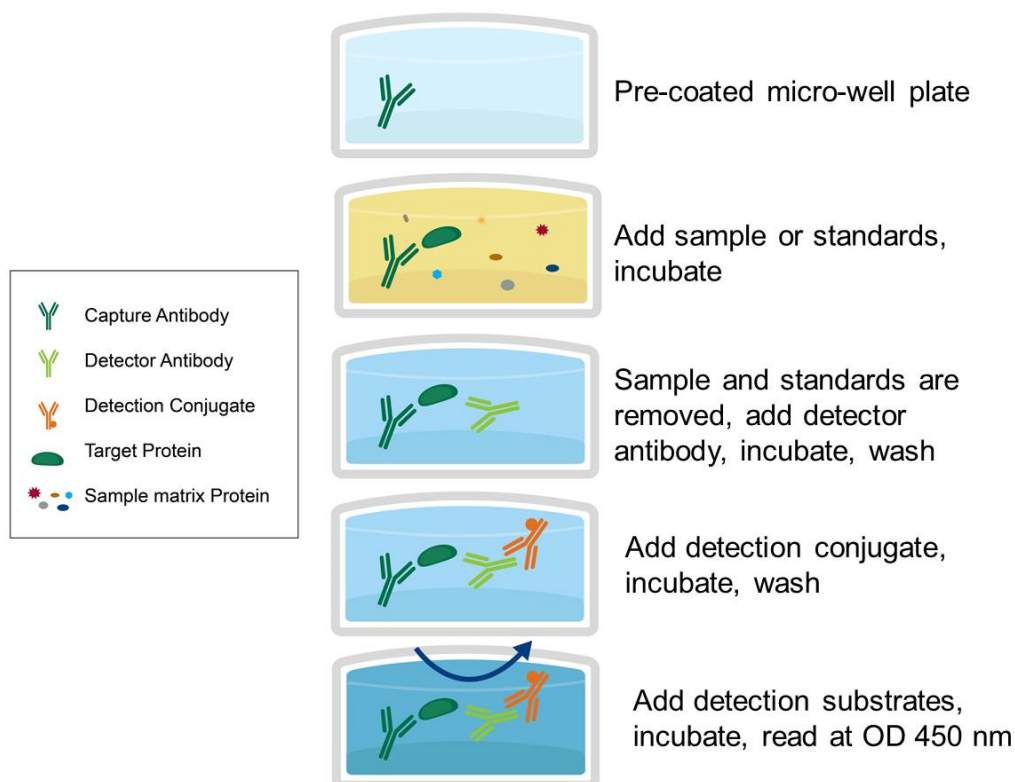
### Target Background

Phosphatidylinositol (PtdIns) phosphatase that specifically hydrolyzes the 5-phosphate of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P<sub>3</sub>) to produce PtdIns(3,4)P<sub>2</sub>, thereby negatively regulating the PI3K (phosphoinositide 3-kinase) pathways. Acts as a negative regulator of B-cell antigen receptor signaling. Mediates signaling from the FC-gamma-RIIB receptor (FCGR2B), playing a central role in terminating signal transduction from activating immune/hematopoietic cell receptor systems. Acts as a negative regulator of myeloid cell proliferation/survival and chemotaxis, mast cell degranulation, immune cells homeostasis, integrin alpha-IIb/beta-3 signaling in platelets and JNK signaling in B-cells. Regulates proliferation of osteoclast precursors, macrophage programming, phagocytosis and activation and is required for endotoxin tolerance. Involved in the control of cell-cell junctions, CD32a signaling in neutrophils and modulation of EGF-induced phospholipase C activity. Key regulator of neutrophil migration, by governing the formation of the leading edge and polarization required for chemotaxis. Modulates FCGR3/CD16-mediated cytotoxicity in NK cells. Mediates the activin/TGF-beta-induced apoptosis through its Smad-dependent expression. May also hydrolyze PtdIns(1,3,4,5)P<sub>4</sub>, and could thus affect the levels of the higher inositol polyphosphates like InsP<sub>6</sub>

### General Specifications

General Specifications	
Range	1.56-100 ng/mL
LOD	<1.56 ng/mL (Derived by linear regression of OD <sub>450</sub> of the Mean Blank + 2xSD)
Specificity	<p>Rat Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1</p> <p><u>UniProt ID:</u> P97573</p> <p><u>GeneID:</u> 10659</p> <p><u>Target Alias:</u> Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1, SH2 domain-containing inositol-5'-phosphatase 1, SH2 domain-containing inositol phosphatase 1, Ship, Ship1, SHIP-1</p>
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

## 2. Assay Summary



## 3. Storage and Stability

- Upon receipt store kit at 4°C for 1 Month or -20°C for 6 Months (with noted exceptions below). Avoid multiple freeze/thaw cycles.

## 4. Kit Components

- The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Inpp5d Microplate	96 Wells (12 x 8 Well strips)	4°C for 1 Months -20°C for 6 Months
Inpp5d Lyophilized Standard	2 x 100 ng	
100X Biotinylated Inpp5d Detector Antibody	1 x 120 µL	
100X Avidin-HRP Conjugate	1 x 120 µL	
Sample Diluent	1 x 20 mL	
Detector Antibody Diluent	1 x 12 mL	
Conjugate Diluent	1 x 12 mL	Store at 4°C
25X Wash Buffer	1 x 30 mL	
Stop Solution	1 x 10 mL	
TMB Substrate	1 x 10 mL	

## 5. Precautions

- Read instructions fully prior to beginning use of the assay kit.

- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

## 6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5  $\mu$ L through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.
- 37°C Incubator (optional)

## 7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.
- Pipetting less than 1  $\mu$ L is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing bilirubin, precipitates or fibrin strands or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
- TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

## 8. Reagent Preparation

- Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.