

## Human/Mouse/Rat PIP5K1A Sandwich ELISA Kit Datasheet

For the quantitative detection of Human/Mouse/Rat PIP5K1A in serum, plasma and cell lysates.

### General Information

Catalogue Number	KE00227
Product Name	Human/Mouse/Rat PIP5K1A Sandwich ELISA Kit
Species cross-reactivity	Human/Mouse/Rat
Range (calibration Range)	0.156-10 ng/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	8394 (Human) / 18720 (Mouse) / 365865 (Rat)
SwissProt	Q99755 (Human) / P70182 (Mouse) / D3ZSI8 (Rat)

### Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 20 ng/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4-af - 30 mL/bottle. For Human serum and plasma	1 bottle	
Sample Diluent PT 1-ef - 30 mL/bottle. For Mouse serum	1 bottle	
Sample Diluent PT 3-ec - 30 mL/bottle. For Rat serum and cell lysates	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

**NB: Do not use the kit after the expiration date.**

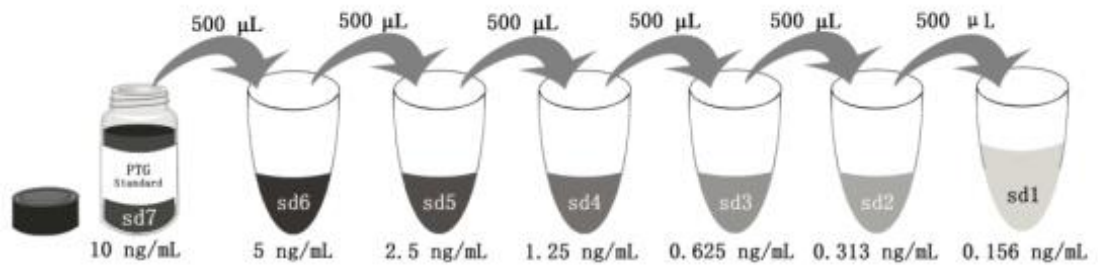
Sample Diluent PT 4-af is for protein standard, human serum and plasma.

Sample Diluent PT 1-ef is for protein standard, mouse serum and plasma.

Sample Diluent PT 3-ec is for protein standard, rat serum, plasma and cell lysates.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 2 mL Sample Diluent PT 4-af ,PT 1-ef or PT 3-ec in protein standard. This reconstitution gives a stock solution of 10 ng/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4-af, PT1-ef or PT 3-ec	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE00227 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The PIP5K1A ELISA kit is to be used to detect and quantify protein levels of endogenous PIP5K1A. The assay recognizes human/mouse/rat PIP5K1A. An antibody specific for PIP5K1A has been pre-coated onto the microwells. The PIP5K1A protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for PIP5K1A is added to detect the captured PIP5K1A protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

## Background

Phosphatidylinositol 4-phosphate 5-kinases (PIP5Ks) play diverse roles in the cellular biology of many organisms, including signal transduction, secretion and vesicular trafficking, and regulation of cytoskeleton assembly(PMID:17688436).

## Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:2 dilution is recommended for human serum ,human plasma and rat serum. Mouse serum is better to be diluted 1:25. Cell lysates is better to be diluted 1:4.

## Safety Notes

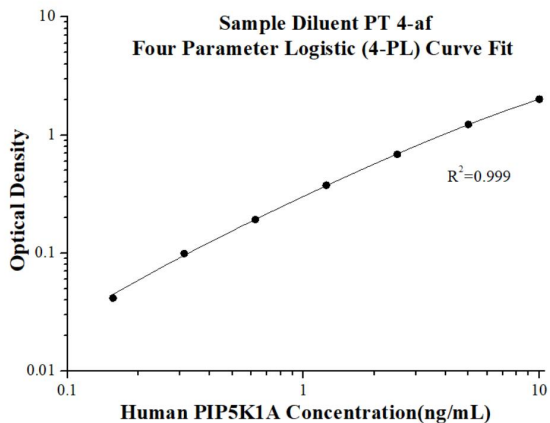
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

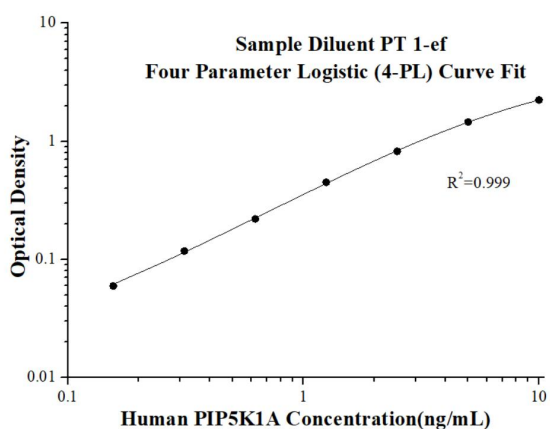
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

## Example data

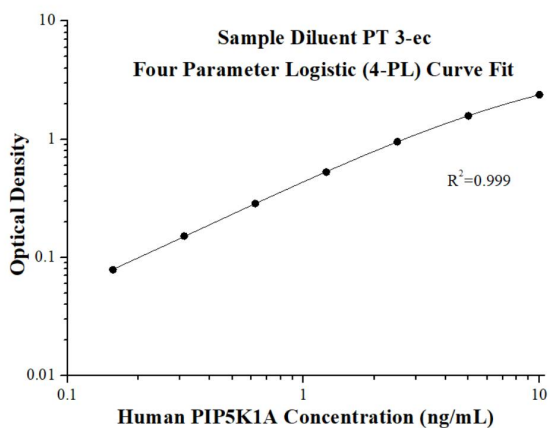
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.078 0.083	0.0805	-
0.156	0.122 0.122	0.122	0.0415
0.313	0.177 0.182	0.1795	0.099
0.625	0.27 0.275	0.2725	0.192
1.25	0.453 0.459	0.456	0.3755
2.5	0.76 0.772	0.766	0.6855
5	1.294 1.321	1.3075	1.227
10	2.126 2.057	2.0915	2.011



(ng/mL)	O.D	Average	Corrected
0	0.064 0.064	0.064	-
0.156	0.125 0.122	0.1235	0.0595
0.313	0.183 0.18	0.1815	0.1175
0.625	0.284 0.284	0.284	0.22
1.25	0.51 0.517	0.5135	0.4495
2.5	0.893 0.879	0.886	0.822
5	1.522 1.516	1.519	1.455
10	2.303 2.292	2.2975	2.2335



(ng/mL)	O.D	Average	Corrected
0	0.083 0.08	0.0815	-
0.156	0.162 0.158	0.16	0.0785
0.313	0.239 0.227	0.233	0.1515
0.625	0.37 0.363	0.3665	0.285
1.25	0.609 0.609	0.609	0.5275
2.5	1.057 1.009	1.033	0.9515
5	1.654 1.664	1.659	1.5755
10	2.47 2.444	2.457	2.3755

## Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	286.2	26.8	9.4
2	20	1,180.2	30.8	2.6
3	20	4,885.1	139.5	2.9

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	270.5	19.9	7.4
2	24	1,212.8	83.7	6.9
3	24	5,531.5	378.8	6.8

## Recovery

The recovery of PIP5K1A spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Range (%)	Average% of Expected
Human plasma	1:2	81-91	85
	1:4	80-98	88
Mouse serum	1:50	76-112	98
	1:100	79-120	106
Rat serum	1:2	78-87	82
	1:4	81-97	89
Cell lysates	1:60	75-95	83
	1:120	83-99	88

## Sample Values

Fourteen individual mouse serum samples were evaluated for the presence of mouse PIP5K1A in this assay.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Mouse serum (n=14)	7.81-38.06	20.3

### cell Lysates:

	PIP5K1A / Total protein (ng/mL)
HepG2 lysateS	5.32
Hela lysateS	1.95
HEK293 lysateS	1.76

## Sensitivity

The minimum detectable dose of Human PIP5K1A is 0.008 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## Linearity

To assess the linearity of the assay, human plasma and Rat serum were spiked with high concentrations of PIP5K1A in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Mouse serum and cell lysates were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

Sample Type		Range (%)	Average% of Expected
Human plasma	1:2	105-111	108
	1:4	119-126	122
	1:8	112-117	114
	1:16	101-103	102
Mouse serum	1:25	-	100
	1:50	115-126	121
	1:100	95-111	103
	1:200	70-75	73
Rat serum	1:2	78-89	84
	1:4	87-104	96
	1:8	99-108	104
	1:16	102-106	104
Cell lysates	1:8	-	100
	1:16	97-108	104
	1:32	97-109	105
	1:64	106-110	108