

## Human APCS Sandwich ELISA Kit Datasheet

For the quantitative detection of Human APCS concentrations in serum, plasma, cell culture supernatants and urine.

### General Information

Catalogue Number	KE00122
Product Name	Human APCS Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6 - 1000 pg/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	325
SwissProt	P02743

### 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 - 1000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100×) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle. For serum, plasma and cell culture supernatants	2 bottles	
Sample Diluent PT 3-ef - 30 mL/bottle. For urine	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 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	

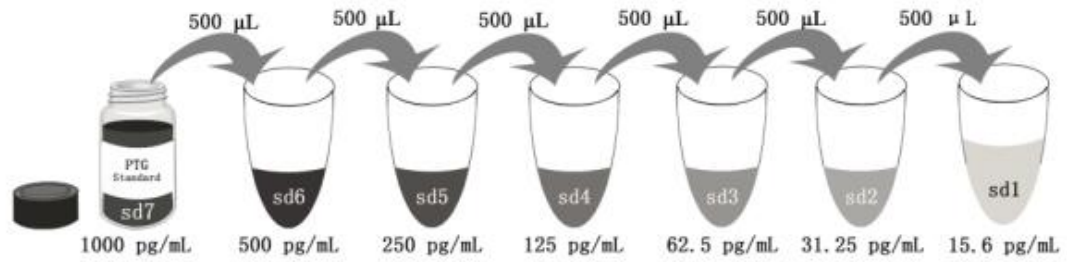
**NB: Do not use the kit after the expiration date.**

**Sample Diluent PT 1-ef** is for protein standard and serum, plasma and cell culture supernatants samples.

**Sample Diluent PT 3-ef** is for protein standard and urine samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 1 mL Sample Diluent PT 1-ef or PT 3-ef in protein standard. This reconstitution gives a stock solution of 1000 pg/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 1-ef or PT 3-ef	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE00122 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The APCS ELISA kit is to be used to detect and quantify protein levels of endogenous APCS. The assay recognizes human APCS. An antibody specific for APCS has been pre-coated onto the microwells. The APCS protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for APCS is added to detect the captured APCS 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

Serum amyloid P component (SAP, also known as APCS), belongs to the family of pentraxins, a superfamily of plasma proteins characterized by their pentameric assembly and calcium-dependent ligand binding. SAP is a constitutive serum protein synthesized by hepatocytes. It is structurally related to C-reactive protein, a major acute-phase reactant in humans. SAP is a calcium-dependent ligand binding protein, which can interact with DNA and histones and may scavenge nuclear material released from damaged circulating cells. SAP can also bind to fibrils in all types of amyloid deposits, and contributes to the pathogenesis of amyloidosis.

## Sample Preparation

The samples may require proper dilution to fall within the range of the assay. The urine is better to be diluted 1:4 or 1:8 before assay, the human serum, plasma is better to be diluted 1:200,000 and 1:2 dilution is recommended for cell culture supernatants.

## Safety Notes

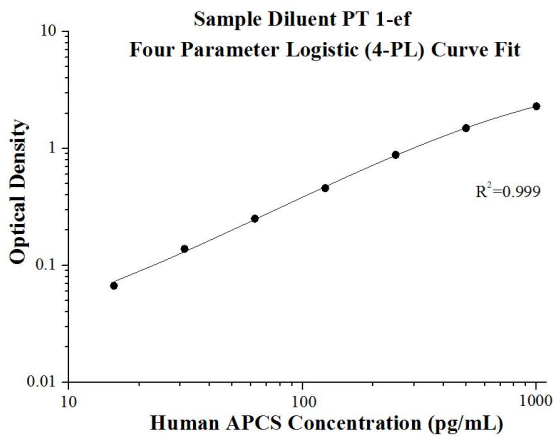
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

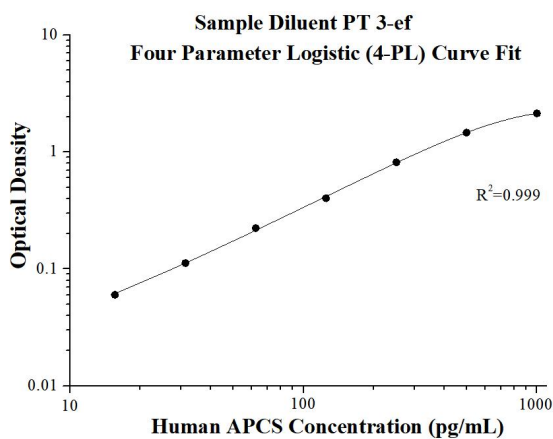
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	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.



(pg/mL)	O.D	Average	Corrected
0	0.032 0.032	0.032	-
15.6	0.089 0.109	0.099	0.067
31.25	0.166 0.175	0.171	0.139
62.5	0.268 0.298	0.283	0.251
125	0.471 0.508	0.490	0.458
250	0.946 0.883	0.915	0.883
500	1.524 1.525	1.525	1.493
1000	2.318 2.337	2.328	2.296



(pg/mL)	O.D	Average	Corrected
0	0.063 0.061	0.062	-
15.6	0.126 0.118	0.122	0.060
31.25	0.167 0.181	0.174	0.112
62.5	0.274 0.297	0.286	0.224
125	0.441 0.488	0.465	0.403
250	0.888 0.870	0.879	0.817
500	1.498 1.556	1.527	1.465
1000	2.236 2.171	2.204	2.142

## 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					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	24.0	2.6	10.9	1	24	28.9	2.9	9.9
2	20	100.4	7.1	7.1	2	24	106.2	10.1	9.5
3	20	432.7	18.2	4.2	3	24	457.7	45.0	9.8

## Recovery

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

Sample Type		Average% of Expected	Range (%)
Human plasma	1:400,000	111	91-120
	1:800,000	107	90-121
Cell culture supernatants	1:2	94	83-119
	1:4	93	81-126
Human urine	1:8	100	75-119
	1:16	106	80-119

## Sample Values

Samples from healthy volunteers were evaluated for APCS in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ug/mL)	Range (ug/mL)
Human plasma (n=16)	53	9-118

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human urine (n=8)	7,011	2,453-18,235

## Sensitivity

The minimum detectable dose of human APCS is 2.6 pg/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, cell culture supernatants were spiked with high concentrations of APCS in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Plasma and urine samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:200,000, the urine samples were initially diluted 1:4)

		Human plasma ( PT 1-ef )	Cell culture supernatants ( PT 1-ef )	Human urine ( PT 3-ef )
1:2	Average% of Expected	100	91	100
	Range (%)	-	76-105	-
1:4	Average% of Expected	89	94	102
	Range (%)	79-95	73-109	93-112
1:8	Average% of Expected	88	97	102
	Range (%)	76-96	92-104	97-108
1:16	Average% of Expected	98	99	95
	Range (%)	88-104	85-124	88-102

## References

1. de Haas CJ, et al. New insights into the role of serum amyloid P component, a novel lipopolysaccharide-binding protein. FEMS Immunol Med Microbiol. 26(3-4):197-202 (1999).
2. Pepys MB, et al. Biology of serum amyloid P component. Ann N Y Acad Sci. 389:286-98 (1982).
3. Familian A, et al. Chromatin-independent binding of serum amyloid P component to apoptotic cells. J Immunol. 167(2):647-54 (2001).
4. Pepys MB, et al. Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. 417(6886):254-9 (2002).