

Human TDP-43 Sandwich ELISA Kit Datasheet

For the quantitative detection of human TDP-43 for human cerebrospinal fluid (CSF) and cell lysates.

General Information

Catalogue Number	KE00009
Product Name	Human TDP-43 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	6.25-400 ng/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	23435
SwissProt	Q13148

Kit Components & Storage

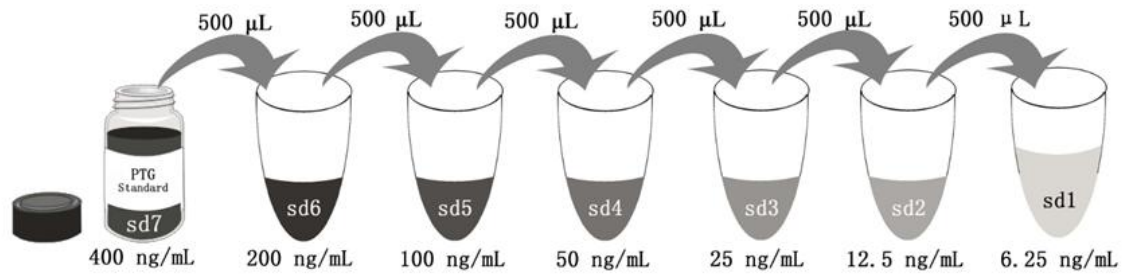
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 400 ng/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µ L/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µ L/vial	1 vial	
Sample Diluent PT 6-e - 30 mL/bottle	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 6-e is for protein standard and samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

*Add 1 mL Sample Diluent PT 6-e in protein standard. This reconstitution gives a stock solution of 400 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 6-e	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00009 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The TDP-43 ELISA kit is to be used to detect and quantify protein levels of endogenous TDP-43. The assay recognizes human TDP-43. An antibody specific for TDP-43 has been pre-coated onto the microwells. The TDP-43 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for TDP-43 is added to detect the captured TDP-43 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

TARDBP was firstly found as a transcriptional repressor that binds to chromosomally integrated TAR DNA and represses HIV-1 transcription. It was also reported to regulate alternate splicing of the CFTR gene. Neumann et al. (2006) found that a hyperphosphorylated, ubiquitinated, and cleaved form of TARDBP, known as pathologic TDP43, is the major disease protein in ubiquitin-positive, tau-, and alpha-synuclein-negative frontotemporal dementia (FTLD-U) and in Amyotrophic lateral sclerosis (ALS). KE00009 is used to quantify TDP-43 level in vivo and it is suitable for sample of CSF and cell lysates. KE00005 is used to quantify TDP-43 level in vivo and it is suitable for sample of serum or plasma.

Sample Preparation

The human cerebrospinal fluid (CSF) samples may require proper dilution to fall within the range of the assay. 1:2 times dilution or no dilution is suggested for human CSF. The cell lysates is better to be diluted 1:20 or 1: 40 times before the assay

Safety Notes

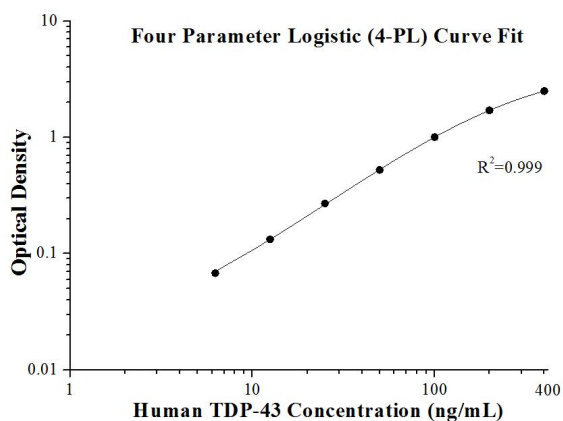
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.082 0.083	0.083	—
6.25	0.154 0.147	0.151	0.068
12.5	0.214 0.218	0.216	0.133
25	0.358 0.347	0.353	0.270
50	0.612 0.601	0.607	0.524
100	1.101 1.072	1.087	1.004
200	1.787 1.793	1.790	1.707
400	2.557 2.606	2.582	2.499

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 (ng/mL)	SD	CV%	Sample	n	Mean (ng/mL)	SD	CV%
1	20	37.2	1.6	4.2	1	24	37.5	3.1	8.4
2	20	73.0	2.0	2.8	2	24	72.9	3.2	4.4
3	20	144.9	6.5	4.5	3	24	153.8	7.2	4.6

Recovery

The recovery of TDP-43 spiked to three different levels in four samples throughout the range of the assay in human cerebrospinal fluid (CSF) and cell lysates were evaluated.

Sample Type		Average% of Expected	Range (%)
Human cerebrospinal fluid	1:2	115	108-128
	1:4	103	95-113
Cell Lysates	1:160	95	85-110
	1:320	96	79-121

Sample Values

Human cerebrospinal fluid samples from healthy volunteers were evaluated for TDP-43 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Sample 1	Sample 2
Human cerebrospinal fluid (n=2)	8.7 ng/mL	60.4 ng/mL

TDP-43 /Total protein (ng/mg)	Hela cell lysates	293 cell lysates
	127.3	145.9

Sensitivity

The minimum detectable dose of human TDP-43 is 0.26 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, samples (human cerebrospinal fluid) were diluted with **Sample Diluent PT 6-e** to produce samples with values within the dynamic range of the assay. (The Cell lysates initially diluted 1:5)

		Human cerebrospinal fluid	Cell Lysates
No dilution	Average% of Expected	90	-
	Range (%)	81-100	-
1:2	Average% of Expected	111	100
	Range (%)	100-118	99-101
1:4	Average% of Expected	90	89
	Range (%)	77-100	81-98
1:8	Average% of Expected	-	86
	Range (%)	-	76-91

References

Neumann M, *et al*, Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006 Oct 6;314(5796):130-3