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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human TLE3 in serum, plasma, cell culture supernatants and urine.

general information

Catalogue Number	KE00048
Product Name	TLE3 ELISA Kit
Species cross-reactivity	Human TLE3
Range (calibration Range)	62.5 - 4000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**7090** (Human)
SwissProt	**Q04726** (Human)

kit components & storage

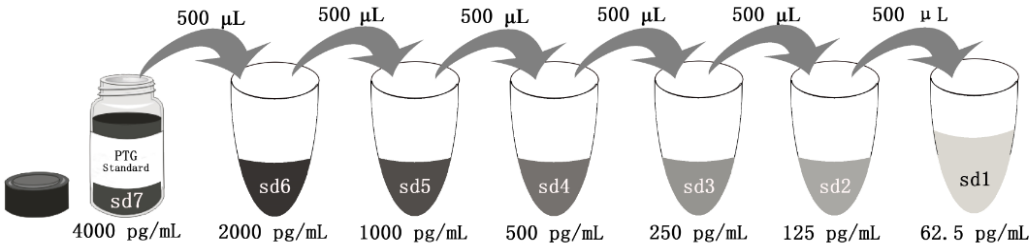
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 8000 pg/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL /vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

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

Sample Diluent PT 1-ef is for Standard and serum, plasma, cell culture supernatants and urine samples.

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

*Add 2 mL Sample Diluent PT 1-ef in Standard, This reconstitution gives a stock solution of 4000 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	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00048 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The TLE3 ELISA kit is to be used to detect and quantify protein levels of endogenous TLE3. The assay recognizes human TLE3. A polyclonal antibody specific for TLE3 has been pre-coated onto the microwells. The TLE3 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for TLE3 is added to detect the captured TLE3 protein. For signal development, horseradish peroxidase (HRP)-conjugated Anti-mouse antibody 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 450nm.

background

Transducin-like enhancer of split 3 (TLE3), also named as KIAA1547 and ESG3, belongs to the WD repeat Groucho/TLE family. TLE3 is a transcriptional repressor that interacts with a chromatin complex acting downstream of adenomatous polyposis coil (APC) and β-catenin in the Wnt pathway. TLE3 is expressed in most β-cells and a subset of other endocrine cell types in the pancreas. TLE3 is first identified as a candidate biomarker of taxane sensitivity in breast cancer in a large screen of candidate immunohistochemical (IHC) classifiers in a community cohort study. TLE3 is a member of the transducin-like enhancer of split (TLE) family of proteins that have been implicated in the tumorgenesis and classification of sarcomas.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

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.

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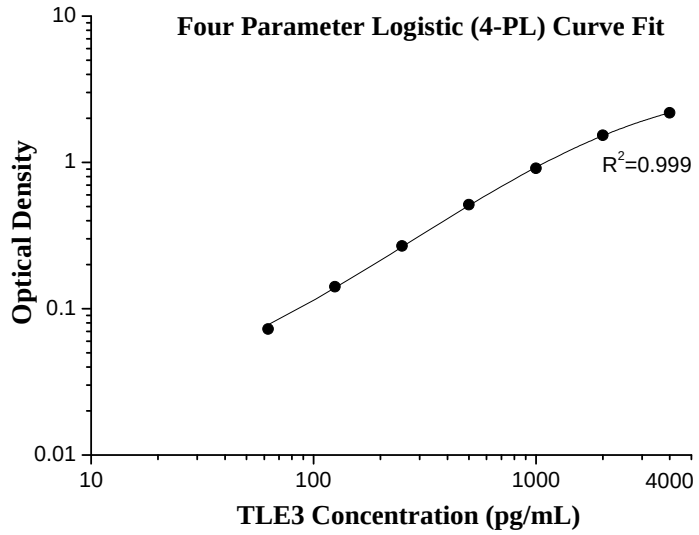
assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 min	4 times	Cover Wells
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
4	TMB Substrate	100 µL	15-30 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.				

typical 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	0.071 0.078	0.0745	—
62.5	0.148 0.146	0.147	0.0725
125	0.21 0.221	0.2155	0.141
250	0.344 0.342	0.343	0.2685
500	0.59 0.586	0.588	0.5125
1000	0.973 0.996	0.9845	0.91
2000	1.583 1.631	1.607	1.5325
4000	2.248 2.268	2.258	2.1835

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	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/ml)	3113.7	945.8	199.8	3006.0	967.4	217.4
SD	98.0	26.8	6.2	141.8	44.6	8.2
CV%	3.1	2.8	3.1	4.7	4.6	3.8

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recovery

The recovery of TLE3 spiked to three different levels in four samples throughout the range of the assay in vrious matrices was evaluated.

Sample Type		Average % of Expected	Range(%)
Citrate plasma	1:4	97	82-126
Cell culture supernatants	1:2	110	97-127
	1:4	102	83-119
Urine	1:2	85	78-93
	1:4	95	83-131

sensitivity

The minimum detectable dose of human TLE3 is 11 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, three samples were spiked with high concentrations of TLE3 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.
(The samples were initially diluted 1:1)

		Citrate plasma	Cell culture supernatants	Urine
1:2	Average% of Expected	94	92	90
	Range(%)	90-98	92-100	84-97
1:4	Average% of Expected	101	98	82
	Range(%)	96-106	95-106	79-85
1:8	Average% of Expected	98	97	85
	Range(%)	89-107	94-106	83-86
1:16	Average% of Expected	107	99	88
	Range(%)	96-117	95-112	83-92

references

1. David E. Metzger1, et al. Ninjurin2, The transcriptional co-repressor Grg3/Tle3 promotes
2. pancreatic endocrine progenitor delamination and β-cell differentiation. Development 139, 1447-1456 (2012).
3. Jennings BH, et al. The Groucho/TLE/Grg family of transcriptional co-repressors. Genome Biol; 9:205（2008）.
4. Ring BZ, et al. Novel prognostic immunohistochemical biomarker panel for estrogen receptor-positive breast cancer. J Clin Oncol;24:3039–47 (2006).

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