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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human NCALD in serum and plasma.

general information

Catalogue Number	KE00028
Product Name	NCALD ELISA Kit
Species cross-reactivity	Human NCALD
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**83988** (Human)
SwissProt	**P61601** (Human)

kit components & storage

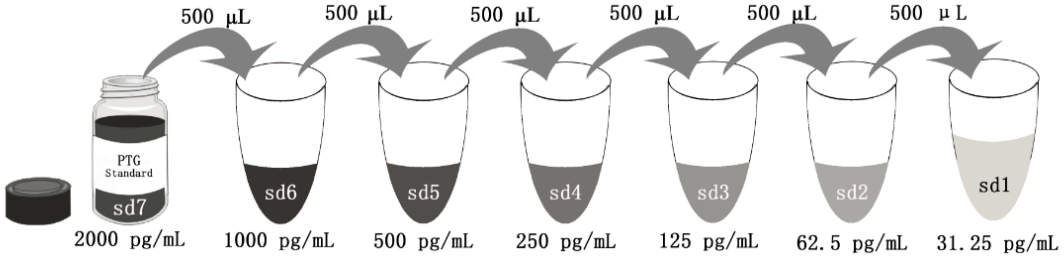
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 4000 pg/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 3-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 3-ef is for Standard and Samples.

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

*Add 2 mL Sample Diluent PT 3-ef in Standard, This reconstitution gives a stock solution of 2000 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 3-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00028 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The NCALD ELISA kit is to be used to detect and quantify protein levels of endogenous NCALD. The assay recognizes human NCALD. A polyclonal antibody specific for NCALD has been pre-coated onto the microwells. The NCALD protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for NCALD is added to detect the captured NCALD 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

NCALD belongs to the recoverin family. And it is a member of the EF-hand calcium-binding protein superfamily. NCALD may be involved in the calcium-dependent regulation of rhodopsin phosphorylation. It plays an important role in the regulation of the neuronal signal transduction process. NCALD is a likely candidate for conferring susceptibility to diabetic nephropathy.

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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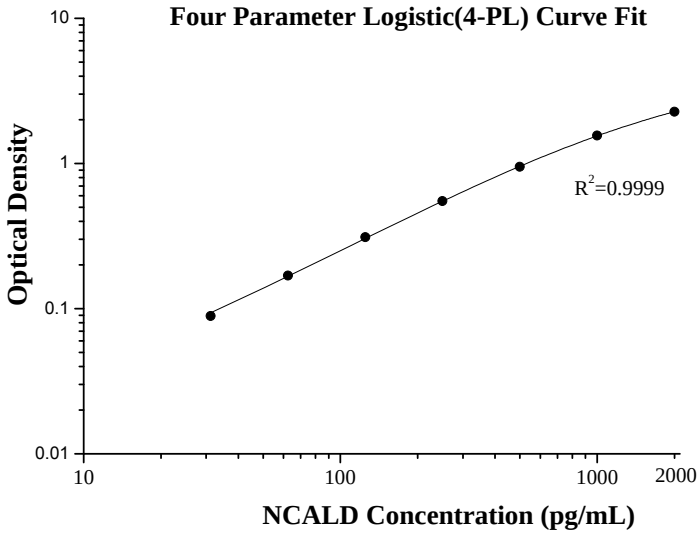
assay procedure summary

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	15-20 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.				

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	0.101	0.0985	——
	0.096		
31.25	0.181	0.1875	0.0895
	0.194		
62.5	0.258	0.2675	0.1695
	0.277		
125	0.416	0.4095	0.3115
	0.403		
250	0.628	0.649	0.551
	0.67		
500	1.036	1.0455	0.9475
	1.055		
1000	1.646	1.6555	1.5575
	1.665		
2000	2.355	2.371	2.273
	2.387		

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)	45.8	813.2	1125.5	57.0	816.9	1183.7
SD	3.2	33.6	44.0	2.6	41.2	56.8
CV%	6.9	4.1	3.9	4.5	5.0	4.8

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recovery

The recovery of NCALD spiked to three different levels in four samples throughout the range of the assay in human plasma averaged 105%, ranging from 96%-125%

sensitivity

The minimum detectable dose of human NCALD is 3 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 NCALD in human plasma and diluted with the appropriate **Sample Diluent PT 3-ef** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

		Citrate plasma
1:2	Average% of Expected	73
	Range(%)	62-84
1:4	Average% of Expected	97
	Range(%)	88-107
1:8	Average% of Expected	116
	Range(%)	107-125
1:16	Average% of Expected	117
	Range(%)	114-120

references

1. Wang, W., et al. W., Molecular cloning, mapping and characterization of the human neurocalcin delta gene (NCALD). Biochim. Biophys. Acta 1518: 162-167, 2001.
2.Kamiyama M, et al. Polymorphisms in the 3' UTR in the neurocalcin delta gene affect mRNA stability, and confer susceptibility to diabetic nephropathy. Hum Genet. 2007 Nov;122(3-4):397-407

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