Human N-cadherin Sandwich ELISA Kit Datasheet

For the quantitative detection of human N-cadherin concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00114
Product Name	Human N-cadherin Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	46.88-3000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	1000
SwissProt	P19022

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 - 3000 pg/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 - 30 mL/bottle. For Human serum and plasma	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 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	

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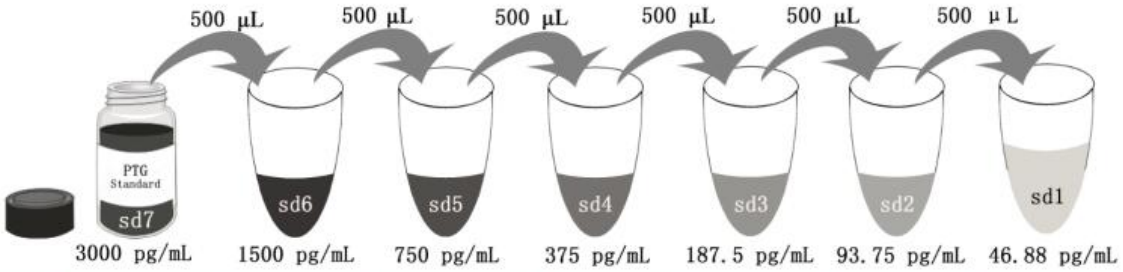
NB: Do not use the kit after the expiration date.
Sample Diluent PT 4 is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 4 in protein standard. This reconstitution gives a stock solution of 3000 pg/mL.



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 4	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00114 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The N-caderin ELISA kit is to be used to detect and quantify protein levels of endogenous N-caderin. The assay recognizes human N-caderin. An antibody specific for N-caderin has been pre-coated onto the microwells. The N-caderin protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human N-caderin is added to detect the captured human N-caderin 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

Cadherins are a family of transmembrane glycoproteins that mediate calcium-dependent cell-cell adhesion and play an important role in the maintenance of normal tissue architecture. N-cadherin (neural cadherin), also known as CDH2 (cadherin 2), is a 130-kDa transmembrane protein and a classical member of the cadherin superfamily which also include E-, P-, R-, and B-cadherins. Expression of N-cadherin has been reported on various cell types including neurons, endothelial cells and cardiac myocytes. N-cadherin has functions in early brain morphogenesis, synaptogenesis and synaptic plasticity. Soluble N-cadherin (sN-CAD) is found in biological fluids, including serum, seminal fluid samples, and urine. It has been shown that significantly higher amounts of serum sN-CAD are present in cancer patients than in persons with no evidence of disease.

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to

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be diluted 1:40 before assay, 1:4 dilution is recommended for cell culture supernatants.

Safety Notes

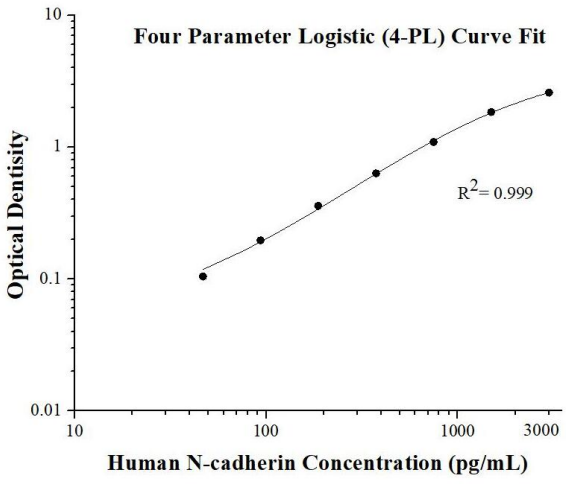
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.026 0.026	0.026	-
46.88	0.131 0.13	0.1305	0.1045
93.75	0.224 0.22	0.222	0.196
187.5	0.384 0.385	0.3845	0.3585
375	0.661 0.655	0.658	0.632
750	1.123 1.115	1.119	1.093
1500	1.858 1.881	1.8695	1.8435
3000	2.592 2.644	2.618	2.592

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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 (pg/mL)	SD	CV%
1	20	1,413.1	48.5	3.4
2	20	395.9	11.8	3.3
3	20	91.9	3.9	4.3

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	1,347.9	72.2	5.4
2	24	348.7	13.6	3.9
3	24	89.0	3.1	3.5

Recovery

The recovery of N-cadherin 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 serum	1:80	96	77-112
	1:160	101	87-117
Cell culture supernatants	1:4	96	86-107
	1:8	96	85-107

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Sample Values

Serum and plasma samples from healthy volunteers (human) were evaluated for N-cadherin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=20)	4,995	190-16,503
Human plasma (n=18)	2,350	99-9,379

cell culture supernatants:

293T were cultured in DMEM supplemented with 10% fetal bonive serum, 2.5 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human N-cadherin, and measured 1,842 pg/mL.

PC-3 were cultured in DMEM supplemented with 10% fetal bonive serum, 2.5 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human N-cadherin, and measured 1,559 pg/mL.

Sensitivity

The minimum detectable dose of human N-cadherin is 5.6 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, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.(The serum samples were initially diluted 1:20,the cell culture supernate were initially diluted 1:2)

		Human serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	107	114
	Range (%)	101-113	107-121
1:8	Average% of Expected	111	111
	Range (%)	104-125	101-121
1:16	Average% of Expected	94	113
	Range (%)	79-116	88-123

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References

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