

## colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human NINJ2 in serum and plasma.

### general information

Catalogue Number	KE00038
Product Name	NINJ2 ELISA Kit
Species cross-reactivity	Human NINJ2
Range (calibration Range)	0.313 - 20 ng/mL
Tested applications	Quantification ELISA

### database links

Entrez Gene	4815 (Human)
SwissProt	Q9NZG7 (Human)

### kit components & storage

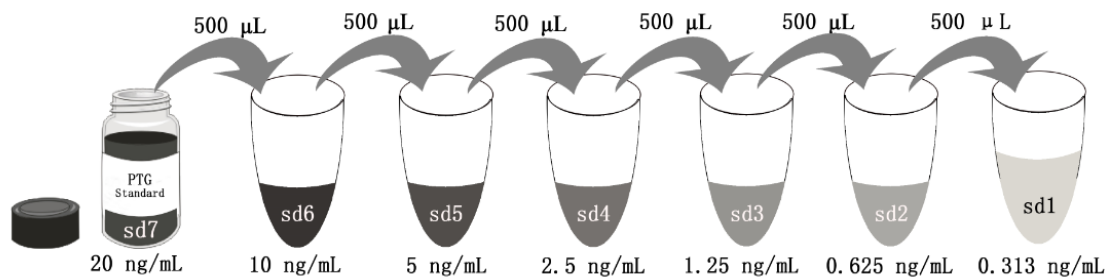
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 40 ng/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ag - 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-ag is for Standard and Samples.

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

\*Add 2 mL Sample Diluent PT 1-ag in Standard, This reconstitution gives a stock solution of 20 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 1-ag	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## product description

KE00038 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The NINJ2 ELISA kit is to be used to detect and quantify protein levels of endogenous NINJ2. The assay recognizes human NINJ2. A polyclonal antibody specific for NINJ2 has been pre-coated onto the microwells. The NINJ2 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for NINJ2 is added to detect the captured NINJ2 protein. For signal development, horseradish peroxidase (HRP)-conjugated 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

NINJ2 (Ninjurin2) is a member of the ninjurin family of adhesion molecules which mediate cell-to-cell and cell-to-extracellular matrix interactions during development, differentiation, and regeneration of the peripheral nervous system. The gene encodes NINJ2 is located on chromosome 12p13. NINJ2 mRNA is widely expressed in adult human tissues, with highest level in bone marrow, followed by peripheral leukocytes, lung, and lymph nodes. In the peripheral nervous system, NINJ2 is expressed constitutively in mature sensory and enteric neurons. The expression of NINJ2 is upregulated after nerve injury in Schwann cells, suggesting that it may promote nerve regeneration. It may also play an important role in the pathogenesis of inflammatory disorder. NINJ2 gene polymorphism is associated with stroke risk, and an association has also been reported between NINJ2 gene polymorphism and Alzheimer's disease risk.

## 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

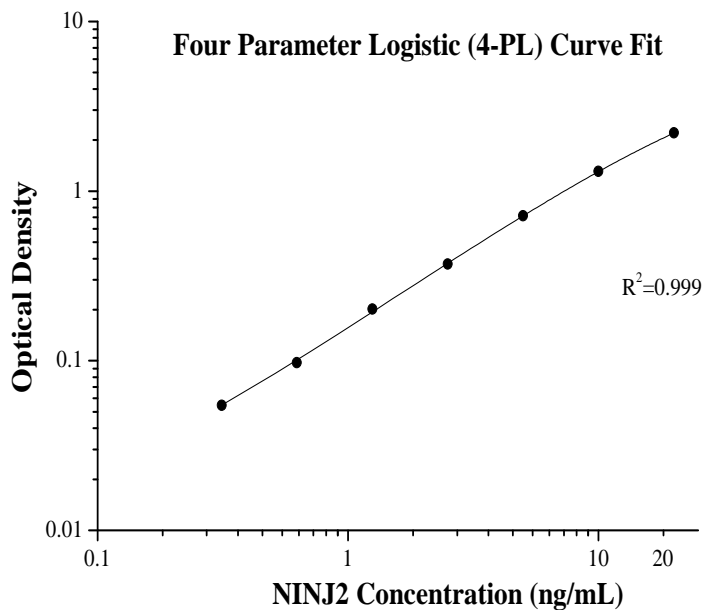
This product is sold for lab research and development use ONLY and not for use in humans or animals.  
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## 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.



(ng/mL)	O.D	Average	Corrected
0	0.039	0.0395	—
	0.04		
0.313	0.094	0.094	0.0545
	0.094		
0.625	0.136	0.137	0.0975
	0.138		
1.25	0.247	0.241	0.2015
	0.235		
2.5	0.403	0.411	0.3715
	0.419		
5	0.737	0.7535	0.714
	0.77		
10	1.328	1.3445	1.305
	1.361		
20	2.217	2.2405	2.201
	2.264		

## 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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/ml)	18.4	8.1	1.7	19.6	7.6	1.5
SD	1.7	0.5	0.1	1.8	0.3	0.1
CV%	9.2	5.7	4.2	9.1	4.2	5.8

## recovery

The recovery of NINJ2 spiked to three different levels in four samples throughout the range of the assay in human plasma averaged 86%, ranging from 72%-102%.

## sensitivity

The minimum detectable dose of human NINJ2 is 0.1 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, three samples were spiked with high concentrations of NINJ2 in human plasma and diluted with the appropriate **Sample Diluent PT 1-ag** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:3)

		Citrate plasma
1:2	Average% of Expected	83
	Range(%)	80-86
1:4	Average% of Expected	96
	Range(%)	89-104
1:8	Average% of Expected	97
	Range(%)	89-107
1:16	Average% of Expected	104
	Range(%)	98-111

## references

1. Araki T, *et al.* Ninjurin2, a novel homophilic adhesion molecule, is expressed in mature sensory and enteric neurons and promotes neurite outgrowth. *J. Neurosci.* 20(1):187-95 (2000).
2. Ikram MA, *et al.* Genomewide association studies of stroke. *N Engl J Med.* 360(17):1718-28 (2009).
3. Lin KP, *et al.* Genetic polymorphisms of a novel vascular susceptibility gene, Ninjurin2 (NINJ2), are associated with a decreased risk of Alzheimer's disease. *PLoS One.* 6(6):e20573 (2011).