Human/Mouse/Rat SNAP25 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00031

Size: 96T

Sensitivity: 6.0 pg/mL

Range: 15.6-1000 pg/mL

Usage: For the quantitative detection of human/mouse/rat SNAP25 concentrations in tissue lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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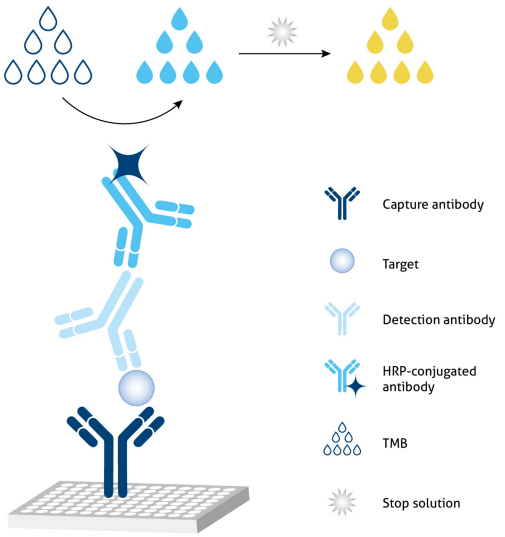
1. Background

Synaptic vesicle membrane docking and fusion is mediated by soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex which is located on the vesicle membrane (v-SNAREs) and the target membrane (t-SNAREs). Other than VAMP2 and STX1A, SNAP25 is another key component of SNARE core complex, and is known to be involved in regulating neurotransmitter release. Its palmitoylation domain is located in the middle of the molecule that contains four cysteine residues and mutation of the cysteines abolishes palmitoylation and its membrane binding activity. As an important presynaptic plasma membrane protein, SNAP25 has been linked to memory and learning by its effect on long term potentiation in the hippocampus, thus playing a critical role in the synaptic function of specific neuronal systems. It has been reported that SNAP25 levels were were significantly decreased in brain tissue homogenates of patients with later stages of Alzheimer's disease compared with the controls, while the levels of SNAP25 were significantly increased in cerebrospinal fluid (CSF) of the group with an Alzheimer's disease biomarker profile than in the group with a control biomarker profile.

2. Principle



Sandwich ELISA structure (HRP conjugated secondary antibody)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody also binds to the analyte. Horseradish peroxidase (HRP)-conjugated secondary antibody binds to the detection antibody. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

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4. Kit Components and 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 - 2000 pg/bottle; lyophilized	2 bottles	
Detection antibody (100×) - 120 µL/vial*	1 vial	
HRP-conjugated antibody (HRP) (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 1-ec - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 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	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

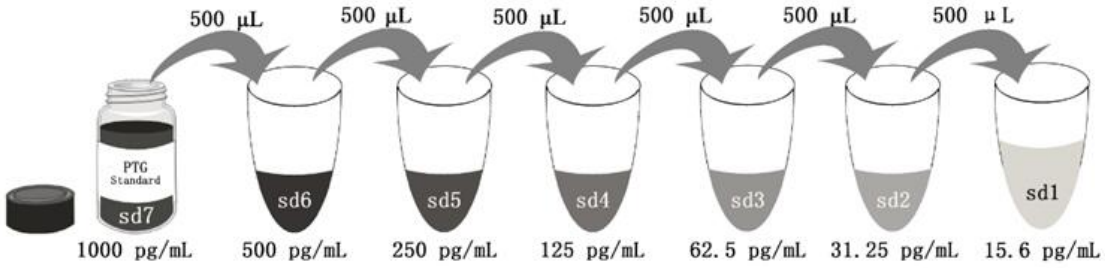
7.3 HRP-conjugated antibody (1X): Dilute 100X HRP-conjugated antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X HRP-conjugated antibody + 990 µL Detection Diluent (Centrifuge the 100X HRP-conjugated antibodyy solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:40 or 1:80 is recommended for tissue lysate.

7.5 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 1-ec in protein standard.



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-ec	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 1 hour at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X HRP-conjugated antibody solution (refer to Reagent Preparation7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C .

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 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.				

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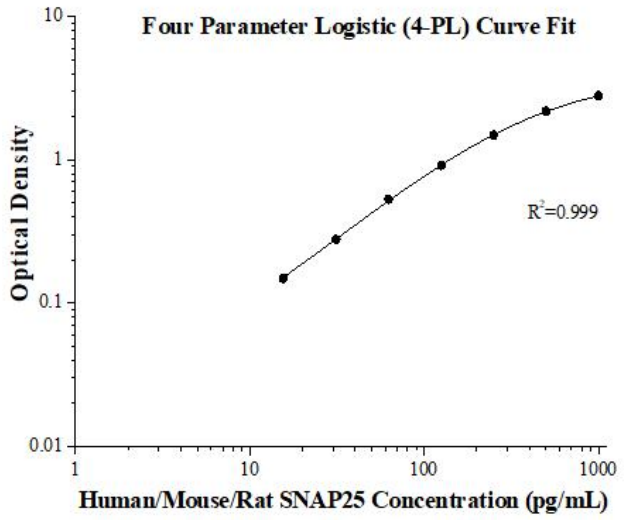
9. Validation Data

9.1 Standard curve

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	0.085 0.082	0.084	-
15.6	0.229 0.235	0.232	0.149
31.25	0.365 0.358	0.362	0.278
62.5	0.618 0.61	0.614	0.531
125	0.994 0.999	0.997	0.913
250	1.588 1.556	1.572	1.488
500	2.297 2.239	2.268	2.185
1000	2.888 2.862	2.875	2.792

9.2 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	36.9	1.1	2.9
2	20	334.6	11.2	3.3
3	20	604.7	27.9	4.6

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	47.1	2.9	6.0
2	24	439.0	30.3	6.9
3	24	841.1	67.0	8.0

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9.3 Recovery

The recovery of human/mouse/rat SNAP25 spiked to three different levels throughout the range of the assay in tissue lysate.

Sample Type		Average% of Expected	Range (%)
Tissue lysate	1:250	92	81-103
	1:500	102	83-119

9.4 Sample values

	Mouse brain lysate	Rat brain lysate
SNAP25 /Total protein (**ng/mg**)	9.6	4.5

9.5 Sensitivity

The minimum detectable dose of human/mouse/rat SNAP25 is 6.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent PT1-ec** to produce samples with values within the dynamic range of the assay.

(The samples were initially diluted 1:20)

		Tissue lysate
1:2	Average% of Expected	93
	Range (%)	85-102
1:4	Average% of Expected	111
	Range (%)	100-119
1:8	Average% of Expected	104
	Range (%)	100-112
1:16	Average% of Expected	98
	Range (%)	86-102

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10. References

1. Chen X., et al. Three-dimensional structure of the complexin/SNARE complex. Neuron 33:397-409 (2002).

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3. Shi L., et al. SNARE proteins: one to fuse and three to keep the nascent fusion pore open. Science. 335(6074):1355-9 (2012).

4. Greber S., et al. Decreased levels of synaptosomal associated protein 25 in the brain of patients with Down syndrome and Alzheimer's disease. Electrophoresis. 20(4-5):928-34 (1999).

5. Brinkmalm A., et al. SNAP-25 is a promising novel cerebrospinal fluid biomarker for synapse degeneration in Alzheimer's disease. Mol Neurodegener. 23;9:53 (2014).

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