SensoLyte® Anti-Mouse/Rat β-Amyloid (1-42) Quantitative ELISA *Colorimetric*

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Catalog #	AS-55554
Kit Size	One 96-well strip plate

This kit is optimized to detect mouse/rat beta-Amyloid (1-42) peptide in mouse/rat brain lysate, cerebrospinal fluid, or plasma. Wells are pre-coated with monoclonal anti-beta-Amyloid (1-42) specific capture antibodies and blocked with a proprietary blocking solution. The amount of mouse/rat beta-Amyloid (1-42) is quantified using ELISA. Ample materials and reagents are provided to perform 96 assays.

- ***Convenient Format***
  - Pre-coated and pre-blocked 96-well strip plate
  - Ready-to-use substrate solution and other assay components
  - One step assay (samples and detection antibodies are added simultaneously)
  - 1 hour assay time at room temperature (excluding incubation)
- ***High Sensitivity***
  - Detects as low as 2 pg/ml (9 fmoles/ml) of mouse/rat beta-Amyloid (1-42) in brain lysate
- ***Broad Dynamic Range***
  - 3.9-250 pg/ml of mouse/rat beta-Amyloid (1-42) peptide

Kit Components and Handling

Component	Description	Quantity
Component A	Mouse monoclonal anti-beta-Amyloid (1-42) coated plate	12 x 8-well strips
Component B	Mouse/rat beta-Amyloid (1-42) Peptide Standard (lyophilized)	1 µg
Component C	1 X Sample Dilution Buffer	60 ml
Component D	10 X Wash Buffer	30 ml
Component E	TMB Color Substrate Solution	10 ml
Component F	Stop Solution	10 ml
Component G	Peptide Standard Reconstitution Buffer	1 ml
Component H	Detection Antibody (Rabbit anti-Mouse/Rat beta-Amyloid-HRP)	50 µl
Component I	1 X Detection Antibody Dilution Buffer	10 ml
Component J	Adhesive Plate Covers	2 pieces

Other Materials Required (but not provided)

- Microplate reader: Capable of reading absorbance at 450 nm
- Rocking platform or shaker
- Strip ejector (to eject strips for future assay if not all strips are used in one experiment)
- Plate washer (optional)

Shipment and Storage

- Kit is shipped on blue ice. Upon receipt, store all kit components at 2-8 °C for up to 6 months
- Reconstituted Peptide Standard (Component B) must be stored at –80 °C in small aliquots

For research use only! Not for diagnostic use!

Introduction

Alzheimer's Disease (AD) is the most common neurodegenerative disorder in elderly people. It has been demonstrated that AD has biological causes and is characterized by the presence of senile plaques and neurofibrillary tangles mainly in cerebral cortex and hippocampus brain regions.[1-5] Beta-Amyloid (1-40) (Aβ40) and beta-Amyloid (1-42) (Aβ42) are the main components of the above plaques; however, other forms of beta-Amyloid peptides are also present. Both peptides are cleaved from the Amyloid Precursor Protein (APP) by β-secretase and γ-secretase enzymes.[2,3,5] Many studies suggest that Aβ42 or/and Aβ43 are required to initiate formation of amyloid plaques and neurofibrills that leads to the neurodegeneration,[1-5] while Aβ40 is less neurotoxic.

The SensoLyte® beta-Amyloid (1-42) Quantitative ELISA Kit (Mouse/Rat) provides a convenient and quantitative assay for determining mouse/rat Aβ42 amount in cell and tissue lysate as well as in body fluids. Compared to other anti-mouse/rat Aβ42 ELISA kits on the market, it takes less time to run this assay. HRP conjugated detection antibody in this kit is added simultaneously with the samples and standards during the assay. This eliminates extra incubation and washing steps and makes this kit a one-step procedure for Aβ42 quantification.

Experimental Protocol

Please Note:

a) Bring kit components to room temperature before starting the assay
b) Spin down all components with volume less than 100 µl before use
c) Mix the Washing Buffer (Component D) to dissolve any precipitated salt

1 ELISA Protocol

1.1 Reconstitute mouse/rat beta-Amyloid (1-42) standard (Component B) with 1 ml of Peptide Standard Reconstitution Buffer (Component G). Allow peptide to hydrate for ten minutes. Mix gently by inverting. Aliquot reconstituted standard in 100 µl per vial (vials are not provided with the kit), label, and save unused standard at –80°C. We recommend using glass vials such as borosilicate for storage of the reconstituted standard to avoid absorption of Aβ42 to plastic surfaces. **Do not reuse reconstituted standard once it is thawed.**

1.2 Arrange and label strips (Component A) based on the number of wells for standards and samples. Although diluted standards and samples can be run as single points, duplicates are recommended. Instructions for preparing cell and/or tissue lysates are provided in the **Appendix** section.
Place unused strips into the supplied bag, seal completely, and store at 4ºC.

1.3 Prepare serial dilution of the Aβ42 standard immediately before use. Refer to the table below.

Step	Concentration, pg/ml	Aβ42 Standard (Component B)	Sample Dilution Buffer (Component C)
1	**1,000,000**	*Prepare as described in 1.1*	
2	10,000	10 µl from step 1	990 µl
3	250	25 µl from step 2	975 µl
4	125	500 µl from step 3	500 µl
5	62.5	500 µl from step 4	500 µl
6	31.25	500 µl from step 5	500 µl
7	15.625	500 µl from step 6	500 µl
8	7.8125	500 µl from step 7	500 µl
9	3.91	500 µl from step 8	500 µl

1.3 Dilute Detection Antibody (Component H)200 fold in Detection Antibody Dilution Buffer (Component I).Prepare 50 µl of the above antibody solution for each well to be run in the assay. One strip requires 0.5 ml of the diluted detection antibody solution.

1.5 Add 100 µl per well of the diluted Aβ42 standards (Starting with Step 3 from 1.3) in duplicates including blank. We recommend diluting plasma at 1:20 dilution ratio and CSF at 1:4 dilution ratio with 1 X Sample Dilution Buffer to avoid sample matrix effects. For brain lysate, it is recommended to use 1:40 dilution ratio. In addition, protease inhibitor cocktail with PMSF must be added to all samples to avoid protein degradation (a recipe example is provided in the **Appendix** section).

1.6 Add 100 µl of the diluted samples into the appropriate wells.

1.7 Add 50 µl of the diluted detection antibody solution (from Step 1.4) into each well to be assayed, apply Adhesive Plate Cover (Component J), and incubate the plate overnight at 4 °C. **Protect wells from the light.**

1.8 Prepare 1 X working wash buffer by diluting the 10 X Wash Buffer (Component D) with deionized H_2O.

1.9 After plate incubation, aspirate the wells and wash them with 350 µl/well of 1 X wash buffer 6-7 times. Allow 20 seconds soaking time before emptying the wells between washes. Pat dry the plate using a paper towel and clean the outside of wells with non-abrasive paper to ensure accurate optical reading.
Insufficient washing will increase background reading.

1.10 Add 100 µl of the TMB Color Substrate Solution (Component E) into each well. Tap plate gently and incubate at room temperature until blue gradient is clearly observed across the wells (5- 15 min). It may be necessary to adjust color development time so that absorbance values fall within the detection range. Samples may require further dilution if Aβ42 concentration is too high.

1.11 Add 50 µl of the Stop Solution (Component F) into each well and tap plate gently (blue color will turn to yellow). Measure absorbance (OD) at 450 nm using a microplate absorbance reader within 20 minutes after adding the Stop Solution.

2. Calculate the concentration of mouse/rat Aβ42 in samples.

2.1 Determine the average values (if replicates are used) for the Aβ42 standard and sample absorbance readings. Plot calibration curve using Linear Regression curve-fit. R^2 should be higher than 0.98. There should be at least 5 standard concentrations in the calculation to ensure statistical significance. The reading for the highest standard concentration (250 pg/ml) can be excluded from the curve if it is too strong.

2.2 Choose absorbance values for the diluted samples that are within the range used in the standard curve, and calculate the concentration of mouse or rat Aβ42 in tested sample(s). Calculated concentrations must be multiplied by the sample dilution factor.

2.3 Typical Aβ42 Standard Curve:
Please note, new standard curve must be generated each time when the assay is run.

Note: 1) *Standards were run in duplicates, blank value was subtracted, and the250 pg/ml point was excluded from the standard curve graph.*
2) *Aβ40 data is shown for cross-reactivity purpose.*

Aβ42, pg/ml	OD @ 450 nm, Aβ42	OD @ 450 nm, Aβ40
250	3.349	0.463
125	2.259	0.115
62.5	1.345	0.034
31.25	0.606	0.021
15.6	0.262	0.000
7.8	0.092	0.007
3.9	0.041	0.01
0	0	0

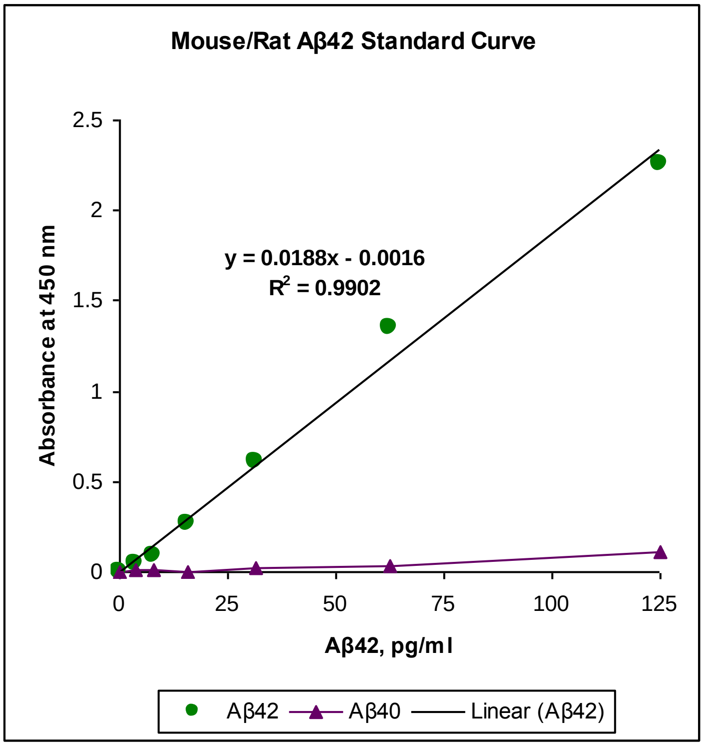

Figure 1. An example of the mouse/rat Aβ42 ELISA standard curve.

3. Kit Performance.

3.1 Aβ42 Recovery Test:

Mouse/rat Aβ42 was added to the diluted mouse brain lysate (1:40 dilution ratio) and assayed using the kit

Specimen	Theoretical Value, (pg/ml)	Measured Value, (pg/ml)	% Recovery
Brain Lysate	10	9.4	94
Brain Lysate	35	33.6	96

Note: *1. Mouse brain lysate was diluted 40 times with Sample Dilution Buffer*
2. Each spiked values was assayed 10 times

3.2 Intra-Assay Variation Test:

Measurement Value, (pg/ml)	Standard Deviation	Coefficient of Variation	n
10	0.4	4.25	10

3.3 Cross Reactivity Test:

Peptide	Cross Reactivity, %
Mouse/Rat Aβ42	100
Mouse/Rat Aβ40	<5
Mouse/Rat Aβ37	<0.1
Mouse/Rat Aβ28	<0.1

Note: *All tested compounds were added at 125 pg/ml concentration.*

Appendix

1. Buffer composition for brain homogenate:

5M Guanidine HCl
50 mM Tris HCl, pH=8.0

Mix 800 µl of the above solution with 100 mg of brain sample in a Dounce homogenizer placed on ice. Homogenize the tissue thoroughly and incubate at room temperature for 3-4 hours with mixing. Guanidine-HCl brain homogenates are stable at 4 °C for several weeks and can be freeze-thawed many times without degradation of beta-Amyloid peptides.
Dilute brain homogenate with "Sample Dilution Buffer" (Component C) for the assay. We recommend 1:40 dilution to start with for mouse brain homogenate. User should determine the optimal dilution factor. Transgenic mouse brain lysates require higher dilution factor due to the high concentration of Aβ42 peptide present in the brain. **Guanidine-HCl concentration higher than 0.125M will result in a significant loss of signal in the assay.**

2. Protease Inhibitor Cocktail Recipe (100 X concentrate)

Aprotinin 0.4 mg
Leupeptin 2 mg
Deionized H_2O 900 µl
Dissolve 0.1 mg of Pepstatin A in 100 µl of methanol and mix with the above solution.
Aliquot and store cocktail at –80 °C.

3. 100 mM PMSF Solution (100 X concentrate)

Dissolve 174 mg of PMSF in 10 ml of pure isopropanol, aliquot, and store at –80 °C.
Add to the protease inhibitor cocktail (1mM final concentration) immediately before use.

References:

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4. Koechling T., et al., Int. J. Alzheimer's Disease, 2010
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