Developing a Highly Sensitive and Economic Assay to Measure beta-Amyloid (1-40) and (1-42) in Body Fluids and Tissue Lysates

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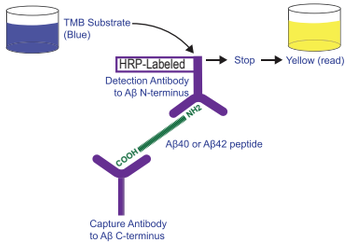
Introduction

Alzheimer's disease (AD), the most common cause of dementia, is characterized by the presence of senile plaques and neurofibrillary tangles, surrounded by damaged neurons. Beta-Amyloid (Aβ) peptides Aβ40 (1-40) and Aβ42 (1-42) are the major components of the above plaques. Many studies suggest that soluble Aβ peptides detected in CSF and blood plasma can serve as promising candidates for biological markers of AD. To further facilitate Aβ detection in biological fluids, we have developed a sensitive and specific one-step ELISA assays to quantify Aβ40 and Aβ42 peptides.

Highly specific mouse monoclonal anti-Aβ40 or anti-Aβ42 capture antibodies and horseradish peroxidase (HRP) labeled rabbit anti-Aβ N-terminal specific detection antibodies were used to develop sandwich ELISA. Assays were further validated for their specificity towards Aβ40 or Aβ42, tested for inter- and intra- assay variability. In addition, we performed recovery tests of Aβ40/Aβ42 peptides from human cerebrospinal fluid (CSF) and human plasma. Transgenic mice brain lysates were also tested. Finally, we employed our Aβ42 assay to study inhibitors of Aβ42 aggregation and compared results with conventional Thioflavin T (ThT) fibrillation test.

Our assays were optimized to achieve recovery of spiked analytes in the range of 100-113% for Aβ 40 and Aβ42 in human CSF and plasma. Statistical analysis demonstrated that assays are precise with internal coefficient of variation (CV) = 1.7-3.1% and CV = 4.18-5.76% for Aβ40/Aβ42 respectively. Intra-assay CVs were computed as 1.9-5.6% for Aβ40 and 5.36-6.12% for Aβ42 kit. Sensitivity of the kits is 2 pg/ml for Aβ40 and Aβ42 as defined by average of negative control reading plus three standard deviations. We have shown that our ELISA kits can be used to screen for Aβ42 aggregation inhibitors. This is especially important when low pico-molar quantities of Aβ peptides are studied. This is more relevant to in vivo events when Thioflavin T assay may not be sensitive enough. Since Aβ aggregation evidently is an essential event in the pathogenesis of AD, the use of new anti-Aβ40/Aβ42 ELISA kits to search for a compound that interrupts aggregation and thus protects against neurotoxicity is of great interest.

Assay Principle



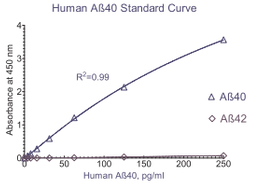
Beta-Amyloid standards and samples are added simultaneously with HRP-labeled detection antibodies into pre-coated anti- Aβ40 or Aβ42 capture antibodies wells. Plate is incubated overnight at 4ºC, washed, and developed with colorimetric tetramethylbenzidine (TMB) substrate. Reaction is stopped with 1M HCl and signal is read at 450 nm using an ELISA plate reader.

Materials and Methods

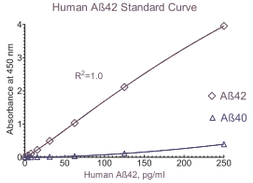
- **SensoLyte® Anti-Human β-Amyloid (1-40) & (1-42) Assay Kits (AnaSpec, Fremont, CA)**
- **Biological Fluids or Samples Tested:**
  Human Cerebrospinal Fluid (CSF) (Fisher Scientific, PA), Human Plasma (Sigma, MO), Mouse Brain Lysates (courtesy of Mayo Clinic, FL)
- **Beta-Amyloid aggregation Inhibitors:**
  3-Hydroxytyramine HCl (Dopamine), Phenol Red, 3-Nitrophenol, o-Vanillin (Fisher Scientific, PA)
- **Aggregation Inhibitors Test:**
  1) Aβ42 was pretreated with HFIP(1,1,1,3,3,3-Hexafluoro-2-propanol), subsequently dissolved in 0.1 M Na bicarbonate buffer (pH=8.5) to 0.25 mg/ml final Aβ42 concentration. Incubation was at 37ºC for 6 days without shaking ±inhibitor at 100μM final concentration for each compound tested
  2) Aggregation assay was performed as follows: 5 μg of each Aβ42 sample pre-incubated with inhibitor or plain buffer was mixed with ThT at 10 μM final concentration. Fluorescence was measured at Ex/Em=440/484 nm using FlexStation 384II (Molecular Devices, Sunnyvale, CA)
  3) Aβ42 samples pre-incubated with inhibitor or Na bicarbonate buffer were diluted to a final concentration of 100 pg/ml and quantified using SensoLyte® Anti-Human β-Amyloid (1-42) ELISA kit

Results

Calibration Curves



Aβ40 ELISA Kit Standard Curve. A typical standard curve for Aβ40 Kit showing low cross-reactivity with human Aβ42 peptide. 4-Parameter Logistics (4-PL) curve fit was used.



Aβ42 ELISA Kit Standard Curve. A typical standard curve for Aβ42 Kit showing low cross-reactivity with human Aβ40 peptide. 4-Parameter Logistics (4-PL) curve fit was used.

Human Aβ40/Aβ42 Recovery

Human plasma was diluted 1:20 and human CSF was diluted 1:4 with Sample Dilution Buffer (SensoLyte® ELISA kit, Component C). Each sample was assayed ten times.

Aβ40

Specimen	Spiked Value, pg/ml	Measured Value, pg/ml	% Recovery
Human Plasma (x20)	10	10.9	109
Human Plasma (x20)	40	42.35	105.8
Human CSF (x4)	10	10.7	107
Human CSF (x4)	25	25	100

Aβ42

Specimen	Spiked Value, pg/ml	Measured Value, pg/ml	% Recovery
Human Plasma (x20)	16	17.23	107
Human Plasma (x20)	25	26.3	105.2
Human CSF (x4)	10	11.31	113
Human CSF (x4)	25	25	100

Assay Precision

Human plasma was diluted at 1:40 ratio with Sample Dilution Buffer and spiked with different amounts of Aβ40 or Aβ42 peptide. Each sample was assayed 10 times using three different kit lots (30 replicates total).

Aβ40

Spiked Aβ40, pg/ml	Inter CV, % 1 plate , n=10	Intra CV, % 3 plates, n=30
5	3.1	5.6
60	1.7	1.9
120	2.5	2.46

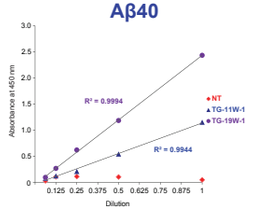
Aβ42

Spiked Aβ42, pg/ml	Inter CV, % 1 plate, n=10	Intra CV, % 3 plates, n=30
5	5.34	6.12
60	5.76	5.45
120	4.18	5.36

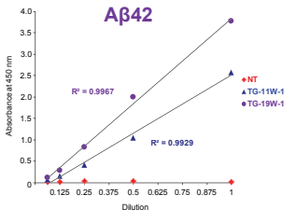
Transgenic Mice Brain Lysate Dilution Test

Transgenic mouse (Tg2576) brain lysate was diluted at 1:400 for 11 weeks old animal (TG-11W-1) and at 1:2,000 ratio for 19 weeks old animal (TG-19W-1). Non-transgenic (NT) mouse brain lysate was diluted at 1:100 ratio. Samples were further serially diluted two-fold and quantified for Aβ40 and Aβ42 using ELISA.

Aβ40



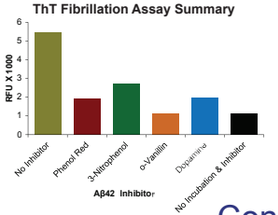
Aβ42



Aggregation Inhibitors Test

ELISA aggregation results are comparable with ThT assay and can provide better sensitivity when inhibitors are screened against low pico-molar quantity of Aβ42.

ThT Fibrillation Assay Summary



Aβ42 Aggregation ELISA Summary

Inhibitor	Expected Aβ42, pg/ml	Measured Aβ42, pg/ml	% Aggregated
No Inhibitor	100	64	36
Phenol Red	100	98	2
3-Nitrophenol	100	100	0
O-Vanillin	100	115	0
Dopamine	100	99	1
No Incubation & Inhibitor	100	100	0

Conclusions

- We have developed highly sensitive and robust assays optimized to measure Aβ40 and Aβ42 in human body fluids and transgenic animal models tissue lysates.
- Our assays provide advantages over competitors' assays by combining a one-step format with high sensitivity reaching 2 pg/ml of Aβ40/Aβ42 detection limit.
- SensoLyte® Anti-Human β-Amyloid (1-40) & (1-42) ELISAs are marked by low coefficient of variation across entire assay dynamic range.
- Presented assays are validated for inhibitors screening in Aβ aggregation test.