PAD-Abeta: Aggregated Form
Version 1

96 well microplate
EIA test kit for beta amyloid (1-40):
Aggregated Form

User Guide
(Version 1.1)

For research use only
1. Overview

The Microsens Sepion Technology™ PAD-Abeta: Aggregated Form EIA Test Kit is an antigen-capture enzyme immunoassay (EIA) on the 96 well microplate format for quantitative detection of aggregation in beta amyloid peptide 1-40. It is designed to provide a sensitive and specific detection of aggregated material in the presence of the normal, monomeric form.

2. Description and Principles

The Microsens PAD-Abeta: Aggregated Form EIA Test Kit uses a proprietary method (US Patent No. 7659076 and other patents worldwide) that allows specific detection of aggregated beta amyloid peptide. A synthetic ligand (one of the Sepion™ range) that binds aggregated A-beta with very high affinity is pre-immobilized on the surface of the 96 microwell Sepion™ Capture Plate. In the test procedure Capture Buffer is first added to the sample and then the mixture is added directly to the ligand-coated wells. The aggregated A-beta present in the sample is bound by the immobilized ligand during the subsequent incubation. The plates are then washed to remove unbound materials, including the normal, monomeric form of A-beta (usually in large excess). The bound aggregated A-beta is detected using BAM10 mouse monoclonal antibody and a rabbit anti mouse secondary antibody conjugated to horseradish peroxidase (HRP). The plate is washed to remove unbound antibody-enzyme conjugate and a peroxidase substrate is added. Colour development in the Capture Plate well is quantitatively related to the mass of aggregated A-beta bound by the ligand.

Interpretation of sample results is based on the absorbance at 450 nm measured in the well.

3. Kit Components

Store all components at 2°C – 8°C.

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Sepion™ Capture Plate – 1 x 96 microwell plate as 8 x 2 microwell strips</td>
</tr>
<tr>
<td>DB</td>
<td>5x Dilution buffer; preserved with sodium azide, 1 x 50 mL</td>
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<tr>
<td>CB</td>
<td>Capture Buffer; preserved with sodium azide, 1 x 5 mL</td>
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<tr>
<td>CAC1</td>
<td>Aggregated A-beta, reactive with Sepion™ Capture Plate; preserved with sodium azide, 1 x 1 mL</td>
</tr>
<tr>
<td>AMCC</td>
<td>HRP-Conjugated Rabbit anti-mouse secondary antibody conjugate concentrate -1x1mL</td>
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<tr>
<td>ABC</td>
<td>Anti A-beta mouse monoclonal antibody (BAM10)</td>
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<tr>
<td>CD</td>
<td>Conjugate diluent: buffer with detergents and protein stabilizers; preserved with Proclin 300, 10 mL</td>
</tr>
<tr>
<td>W</td>
<td>10X wash solution - preserved with sodium azide, 2 x100 mL</td>
</tr>
<tr>
<td>T</td>
<td>TMB substrate - 10 mL</td>
</tr>
<tr>
<td>S</td>
<td>Stop solution – 10 mL</td>
</tr>
</tbody>
</table>

4. Materials and Equipment Required (Not Provided)

- Precision pipettes and multi-channel pipettes suitable for delivering between 25 and 200 µL. Reagent volumes listed in the Test Procedure require pipette precision of ±5%
- Graduated cylinders for wash solutions
- Reagent-dispensing trays
- 96-well plate reader (equipped with 450-nm filters) and plate washer

5. Preparation of Reagents

a) Wash Solution
The wash solution concentrate should be brought to room temperature (18°C–25°C) and mixed to ensure dissolution of any precipitated salt. The wash concentrate must be diluted 1:10 with distilled or deionized water before use (e.g. 40 mL of concentrate plus 360 mL of water per plate to be assayed).

b) Positive Control
Positive control is supplied in liquid form ready to use.

c) Sample Preparation
The binding of aggregated A-beta to Sepion ligand is of a mixed mode character and involves an element of charge interaction. Thus, samples for assay should be diluted to have a final ionic strength of 10 mM or less. A Dilution buffer, DB is provided in the kit for this purpose. The Dilution buffer is supplied at 5x strength and should be diluted in distilled water before use. Ideally, the test sample should be tested at a final concentration of 10-100 µg/ml (after dilution in Dilution Buffer).

The kit detects a range of aggregate sizes. Prior to testing, in order to estimate the sizes of aggregates being detected, samples can be diluted in 1x Dilution buffer and filtered through filters of various micron pore size. The effect of filtration on the assay signal is an indication of the size of aggregate present in the sample.

IMPORTANT. Prior to testing, add 25µL Capture Buffer, CB supplied to each 100µL of sample (or each 100 µl of sample diluted in Dilution buffer) and mix.

d) HRP-Conjugated anti-mouse antibody Solution
The HRP-conjugated Rabbit anti-mouse antibody solution is prepared by diluting the conjugate concentrate (AMCC) into the conjugate diluent (CD) at 1:10 (Use within 4 hours)

e) Anti A-beta mouse monoclonal antibody
Prepared by diluting into the conjugate diluent (CD) at 1:10 (Use within 4 hours)

All reagents should be at room temperature (18°C–25°C) before use. Before starting the test, prepare the solutions to be used in the assay. Mix all reagents by gentle swirling. Positive Controls should be tested in duplicate. It is recommended that a cover is used on the microplate for the duration of the antigen capture, conjugate and substrate incubations.

6. Storage of Prepared Reagents

HRP-Conjugated anti-mouse Antibody Solution: Four hours at 18°C–25°C
Wash solution: One week at 18°C–25°C
Store any unused portion of plates in a dark, desiccated, sealed container.
7. Test Procedure

1. Set up a template indicating where the sample positions are located on the Seprion™ Capture Plate. Reserve wells A1, B1, C1, D1 for the kit controls. E1 and F1 should be used as conjugate control wells.

2. Dispense Negative Control (100 μL) if used into wells A1 and B1. Dispense Positive Control (100 μL) into wells C1 and D1. Dispense 1x wash solution into wells E1 and F1.

3. Dispense 100 μL of test sample with added Capture buffer (see Sample preparation, section 5c, above) into the appropriate wells of the Seprion™ Capture Plate.

4. Antigen capture: Incubate the plate for 1 hour at room temperature (18°–25°C).

5. Wash: Aspirate or tap out the contents of the wells. Wash the wells by filling the wells three times with 1X wash solution. Aspirate or tap out and fill the wells after each wash. Aspirate or tap out the final wash.

6. Detection antibody binding: Add 100 μL of the prepared anti-A-beta antibody solution to all the wells and incubate for 60–75 minutes at room temperature (18°–25°C). No shaking required.

7. Wash: Aspirate or tap out the contents of the wells. Wash the wells by filling the wells five times with 1X wash solution. Aspirate or tap out and fill the wells after each wash. Aspirate or tap out the final wash.

8. Secondary antibody conjugate: Add 100 μL of the prepared rabbit anti mouse HRP secondary antibody conjugate solution to all the wells and incubate for 60–75 minutes at room temperature (18°–25°C). No shaking required.

9. Wash: Aspirate or tap out the contents of the wells. Wash the wells by filling the wells five times with 1X wash solution. Aspirate or tap out and fill the wells after each wash. Aspirate or tap out the final wash.

10. Enzyme substrate: Add 100 μL of the TMB substrate to all wells. Incubate for 30 minutes (±1 minute) at room temperature (18°–25°C); protect the plate from direct light.

11. Quench the HRP-mediated color development by adding 100 μL of acid stop solution to the plate. The plate can be retained for up to 30 minutes in the dark at 18°–25°C prior to reading the absorbance in each well.

12. The signal for each sample is determined by reading the absorbance of the microwells at 450 nm.

For estimation of aggregate quantity, subtract the mean conjugate control signal from the test signal and see the standard curves at the end of this document.

Note: the cut-off for reading the signal is 0.2 after subtraction of the mean conjugate control signal. Samples that generate signals below 0.2 either have no aggregation or the aggregates are at levels too low to measure. In order to quantitate signals above 2.7 OD450nm, further dilutions of the product should be performed and these dilutions tested in the ELISA.

Customers can generate their own standard curves for their own products by following the protocol found at http://bit.ly/Rs1zBY

Plate Validity

For the assay to be valid, the Negative Control mean signal (NCx) must have a value less than 0.120 and the positive control mean signal (PCx) must have a value ≥0.400.

Precautions

- Do not expose the TMB substrate to strong light or oxidizing agents. Use clean or disposable plasticware for dispensing TMB.
- Care should be taken to avoid contamination of kit components. Do not use components past their expiration dates, and do not intermix components from different kit lots.
- Some kit components contain sodium azide as a preservative (see description of kit components). Care should be taken to prevent contaminating the HRP-conjugated anti mouse antibody conjugate with azide-containing solutions.
- Store all reagents at 2°–8°C. Bring reagents to room temperature (18°–25°C) prior to use, and return to proper storage temperatures after use (see Storage of Prepared Reagents).
- Use separate dispensing trays for each reagent used in the assay. Avoid cross-contamination of the TMB substrate with the diluted conjugate solution. Do not pour unused TMB solution back into the bottle.
- Do not allow microwell plates to sit more than 5 minutes between wash steps and the addition of reagents.

Safety Information

- The TMB substrate may irritate skin and eyes. Avoid direct contact. Stop solution contains 0.5M sulphuric acid.

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