Pinpoint Molecular Ltd
Amyloid Extraction Kit
(AE-100)

Instructions for Use
For research use only

Version 05

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1. INTENDED USE

The Amyloid Extraction Kit enables the extraction of amyloid-type protein aggregates from samples. Amyloid-forming proteins that have been successfully extracted include prion protein, beta-amyloid, tau, alpha-synuclein, huntingtin, and p53 from multiple sample types such as brain, muscle, plasma, CSF and cell culture.

2. PRINCIPLES OF THE PROCEDURE

The Amyloid Extraction Kit is based on magnetic bead technology. The magnetic resin beads only bind aggregated amyloid-type proteins and do not bind the monomer. Aggregated amyloid-type proteins are captured by the magnetic resin beads which are then washed in wash buffer and eluted from the beads into elution buffer. Eluted amyloid-type proteins can be then detected by an ELISA which is specific for the amyloid-type protein of interest.

3. KIT COMPONENTS

Amyloid Extraction Capture Buffer 1 for plasma and CSF (AECB1-100)
Amyloid Extraction Capture Buffer 2 for homogenates and protein aggregates formed *in vitro* (AECB2-100)
Amyloid Extraction Magnetic Resin Beads (AEMB-100)
Amyloid Extraction Wash Buffer 1 (AEWB1-100).
Amyloid Extraction Wash Buffer 2, 10x concentrate (AEWB2-100). Dilute to 1x in distilled water before use.
Amyloid Extraction Elution Buffer A (AEEA-100)
Amyloid Extraction Elution Buffer B (AEEB-100)

4. STORAGE & HANDLING

- The Amyloid Extraction Kit is shipped at 15-25°C.
- Upon arrival store the Amyloid Extraction Capture Buffer 2 and the Amyloid Extraction Wash Buffer 1 at 4-8°C. All other components can be stored at 15-25°C in a light-free environment.
- The kit should not be used beyond the expiry date displayed on the packaging label, after which the kit should be safely discarded in line with local regulations.
- If the kit components or protective packaging is damaged upon receipt, please contact Pinpoint Molecular Ltd.
- Before use, check that all reagents are free of crystallisation. If crystals are

present, warm at 37°C with mixing until crystals are dissolved.

- The Amyloid Extraction Kit is for professional use only and should be handled by trained healthcare and laboratory professionals operating in accordance with Good Laboratory Practice and local laboratory regulations.
- Users should always wear disposable gloves when handling the kit components.
- Users should consult the safety data sheet (SDS) before use.

5. MATERIALS AND DEVICES REQUIRED BUT NOT PROVIDED

- A magnetic rack that holds 1.5/2 ml microfuge tubes.
- A heating block at 80°C that can hold 1.5/2 ml microfuge tubes.
- A repeat pipette (stepper) that can deliver multiple volumes of 1.0 ml to make washing easier.
- A vacuum source may be utilised to aid the removal of liquid from tubes.
- A vortexer.
- A shaking platform.

6. PROCEDURE

Each sample type may have a different preparation procedure prior to extraction of aggregated amyloid-type proteins, see below.
Each sample type may have a different extraction protocol, see sections 6.1 and 6.2 below.

PREPARATION OF SAMPLES BEFORE EXTRACTION

A. **Plasma.** Plasma should be prepared from EDTA or citrated blood. The use of heparin is not recommended. Use protocol 6.1 below for capture.
B. **CSF.** CSF can be used directly. Use protocol 6.1 below for capture.
C. **Tissue homogenates e.g. brain or muscle or cell culture homogenates.** The amount of homogenate tested has to be determined experimentally; we recommend to start by extracting and testing 1 mg of homogenate. Use protocol 6.2 below for capture.
D. **Protein/peptide aggregates formed *in vitro*.** Protein or peptide aggregates of the protein of interest can be formed *in vitro*. For different methods of aggregation, please see our website www.pinpointmolecular.com/how-to-make-peptide-aggregates. The amount of protein to be extracted and tested must be determined empirically; we recommend to start by extracting and testing nanogram (ng) amounts of aggregate. Use protocol 6.2 below for capture.

6.1 CAPTURE OF AGGREGATED AMYLOID-TYPE PROTEINS FROM PLASMA OR CSF

We recommend this protocol for the capture of small aggregates or oligomers such as might be found in plasma or CSF.

1. For each sample to be tested, add 200 µl of sample (plasma or CSF) to a 1.5 ml microfuge tube.
2. To each tube, add 50 µl of Amyloid Extraction Capture Buffer 1 and mix gently. Leave the tubes for 10 min at room temperature.
3. To each tube add 540 µl of distilled water and 10 µl of Amyloid Extraction Magnetic Resin Beads (ensure that the beads in the reagent bottle are fully suspended before use by shaking the bottle vigorously for at least 5 seconds). Mix the samples gently with the water and beads and place on a shaking platform (do not shake vigorously; only shake at a speed that ensures that the beads are kept in suspension). Leave shaking for 30 min. If a shaking platform is not available, vortex gently every 3 min for 30 min.
4. After step 3, proceed below to step **6.3 Washing of the Magnetic Beads**.

6.2 CAPTURE OF AGGREGATED AMYLOID-TYPE PROTEINS FROM HOMOGENATES

We recommend this protocol for the capture of larger aggregates or amyloid fibrils such as might be found in tissue homogenates or protein and peptide homogenates formed *in vitro*.

1. For each sample to be tested, add 100 µl of homogenate diluted in water or PBS to a 1.5 ml microfuge tube.
2. To each tube, add 220 µl of distilled water and 80 µl of Amyloid Extraction Capture Buffer 2 and mix gently.
3. To each tube add 10 µl of Amyloid Extraction Magnetic Resin Beads (ensure that the beads in the reagent bottle are fully suspended before use by shaking the bottle vigorously for at least 5 seconds). Mix the samples gently with the beads and place on a shaking platform (do not shake vigorously; only shake at a speed that ensures that the beads are kept in suspension). Leave shaking for 15 min. If a shaking platform is not available, vortex gently every 3 min for 15 min.
4. After step 3, proceed below to step **6.3 Washing of the Magnetic Beads**.

6.3 WASHING OF THE MAGNETIC BEADS CONTAINING THE CAPTURED AGGREGATES

Amyloid Extraction Wash Buffer 1 is supplied ready diluted but Amyloid Extraction Buffer 2 is supplied as a 10x concentrate and needs to be diluted 10-fold before use.

1. Place the tubes in a magnetic rack and allow the beads to be collected at the side of the tubes, then carefully remove the liquid without disturbing the beads. A gentle vacuum aspirator may be used to speed up the process. Ensure any residual liquid on the underside of the lid is removed as well.
2. Remove the tubes from the magnetic rack and add 0.25 ml of Amyloid Extraction Wash Buffer 1 to each tube. Gently vortex the tubes to resuspend the beads. If clumps of beads persist, use a clean tip to resuspend the beads by pipetting up and down.
3. Place the tubes back in the magnetic rack and allow the beads to be collected at the side of the tubes, then carefully remove the liquid without disturbing the beads.
4. Remove the tubes from the magnetic rack and add 0.5 ml of Amyloid Extraction Wash Buffer 2 (diluted to 1x from the 10x stock) to each tube. Gently vortex the tubes to resuspend the beads. If clumps of beads persist, use a clean tip to resuspend the beads by pipetting up and down.
5. Place the tubes back in the magnetic rack and once the beads are collected at the side of the tubes carefully remove the liquid.
6. Remove the tubes from the magnetic rack and add 0.25 ml of Amyloid Extraction Wash Buffer 2 (diluted to 1x from the 10x stock) to each tube. Gently vortex the tubes to resuspend the beads. If clumps of beads persist, use a clean tip to resuspend the beads by pipetting up and down.
7. Place the tubes back in the magnetic rack and once the beads are collected at the side of the tubes carefully remove as much liquid as possible, including any liquid on the sides of the tubes. A quick pulse of the tubes in a microfuge to bring residual wash to the bottom of the tube might be helpful here.

6.4 ELUTION OF THE CAPTURED AGGREGATES FROM THE MAGNETIC BEADS

1. Remove the tubes containing the washed magnetic resin beads from the magnetic rack. Add 30 µl of Amyloid Extraction Elution Buffer A to the bottom of each tube and resuspend the beads by gentle vortexing.
2. Heat the tubes at 80°C for 3 min.
3. After heating, immediately place the tubes in a magnetic rack. Do not resuspend the beads in the eluate.
4. Remove the eluate to a fresh labelled tube.
5. Add 10 µl of Amyloid Extraction Elution Buffer B to the eluate and mix by gentle

vortexing.

6. The eluate may now be analysed by testing in an appropriate test e.g. an ELISA for the protein of interest. Follow the supplier's instructions, for example, the eluate should be added to the sample diluent supplied with the supplier's kit.

LIMITATIONS

- The Amyloid Extraction Kit has been validated for prion protein, beta-amyloid, tau, alpha-synuclein, huntingtin, and p53 from multiple sample types such as brain, spleen, solid tissue, and plasma.
- The procedures and processes described in this document should be strictly adhered to in order to obtain correct test results.
- Deviation from this protocol and Good Laboratory Practice may result in failure, or error of results.
- This kit is validated for professional use only and should be performed by qualified healthcare professionals and trained laboratory personnel.
- All samples should be handled assuming they are infectious and following the World Health Organisation Guidance on Laboratory Biosafety issued on 12th of February 2020 (Document Reference WHO/WPE/GIH/2020.1).

7. TECHNICAL SUPPORT

For technical support in relation to the Amyloid Extraction Kit please contact us at:

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