Seprion™-PAD

Tools for Protein Aggregation Disease Diagnostics

Microsens Seprion Technology™

CalBioreagents

PAD-Beads

Magnetic bead based sample preparation system for Protein Aggregation Diseases

Rapid and simple sample preparation for the detection of amyloidProtein Aggregation Diseases.

Creutzfeldt Jakob Disease (CJD and other prion diseases), Alzheimer's, Parkinson's and Huntington's Diseases

Simple and effective capture of the disease associated protein aggregates

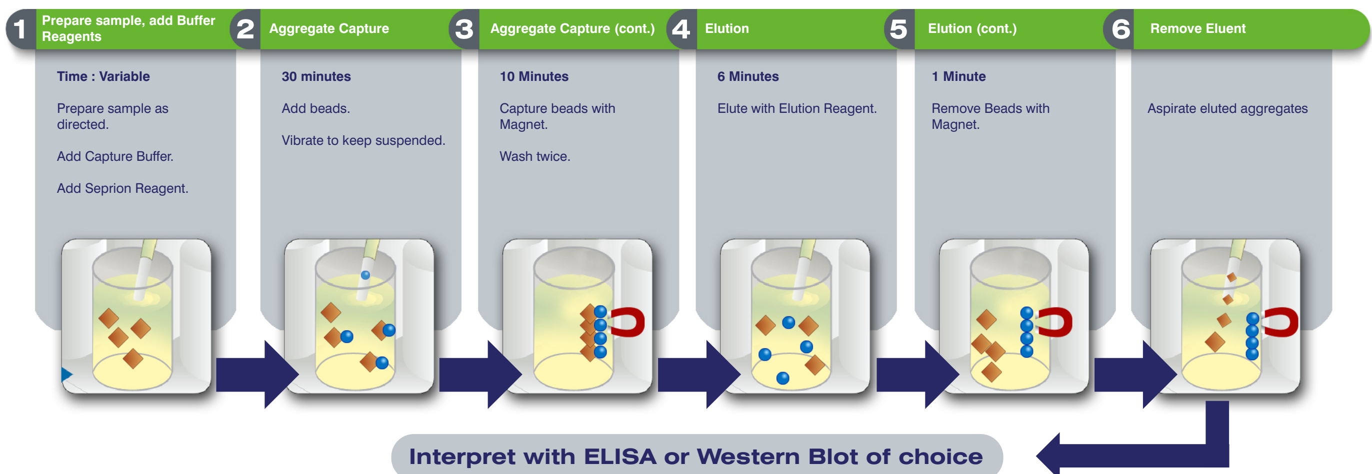
Simply elute into ELISA or Western Blot of choice to determine presence of aggregated protein

Simple

• Homogenize sample • Dilute sample • Capture on beads • Elute into ELISA or Western Blot

Robust

• All room-temperature • Not time sensitive • Less risk of false +ve & -ve results



Proven established technology

Chosen by the UK Health Protection Agency for the UK vCJD Tonsil Study

Microsens Seprion Technology™ – at the heart of the world's fastest growing TSE Kits

(The Idexx HerdChek BSE, BSE/Scrapie and CWD kits)

For research use only

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PAD-Beads

Study the aggregated proteins associated with a wide range Protein Aggregation Diseases

The Seprion Sample Preparation System for Protein Aggregation Diseases, **PAD-Beads**, enables the specific study of the abnormal pathogenic forms of: prion, ß-amyloid & tau, α-synuclein, and huntingtin proteins in CJD, BSE, Scrapie, CWD, Alzheimer's, Parkinson's and Huntington's Disease*.

Without this separation it is difficult if not impossible to study the abnormal forms of the proteins. **PAD-Beads** is an ideal front-end separation technique that can be combined with a back-end commercial or in-house ELISA or Western blot analysis. Research applications include the study and monitoring of Protein Aggregation Diseases in human and animal samples and drug screening in vitro and in vivo.

PAD-Beads uses a version of Microsens's unique Seprion ligand technology (**Seprion-PAD**) that captures and easily separates the abnormal (aggregated) protein present in a wide and expanding range of amyloid Protein Aggregation Diseases from their normal counterparts, whether protease resistant OR not. (Lane et al. 2003. Clinical Chemistry 49:1774-1775, Patent Pending WO/03/073106).

The **Seprion-PAD** ligand is the critical core component of:

•The Idexx Herdcheck (http://www.idexx.com/production/ruminant/ruminant8.jsp) series of commercial tests for BSE, scrapie and CWD that have been extensively evaluated and have received EU and USDA approvals

AND

•The CJD tests being used by the Health Protection Agency for the epidemiologoical study of CJD in tonsils (http://www.hpa.org.uk/infections/topics_az/cjd/tonsil_archive.htm).

*__PAD-Beads__ is likely to work with other Protein Aggregation Diseases including: amylin in Diabetes Type II; crystalline in cataracts; antibody light chain, serum amyloid A, and ß2-microglobulin in amyloid including primary and secondary systemic amyloidosis; and superoxide dismutase 1 in amyotrophic lateral sclerosis but these diseases remain to be tested with **PAD-Beads**.

PAD-Beads Protocol

Capture

For each separation, start with 200µl of sample that has been processed as described above. If the volume is less than 200µl make up the volume with distilled water.

1. To the 200µl of sample in a microtube and add 100µl of Capture buffer, mix thoroughly.
2. Add 100 µl Seprion reagent and mix.
3. Add 100µl beads (carefully resuspend the beads prior to use).
4. Shake by vibration (so that the beads do not settle) for 30 min at room temperature.
5. Capture the beads on a magnet and remove the liquid.
6. Add 1ml Wash buffer 1 to the beads and resuspend by vortexing.
7. Recapture the beads and wash twice with 1 ml Wash buffer 2.
8. Capture the beads and remove the liquid.
9. Remove the tubes from the magnet and pulse spin in a microfuge. Place back on the magnet and remove the last dregs of liquid.

Elution

Elution method of choice for ELISA analysis

The captured protein can be eluted and analysed by ELISA for the protein of interest.

1. Add 10 µl of ELISA Elution buffer 1, resuspend the beads and shake for 5 min.
2. Add 10 µl of ELISA Elution buffer 2 and mix.
3. Place the tubes on a magnet to capture the beads.
4. Remove the liquid and analyse by ELISA. The eluted liquid can be mixed with the sample diluent supplied with most commercial or in-house ELISAs and tested directly.

Elution method for Polyacrylamide gel electrophoresis (PAGE)

The captured protein can be eluted and analysed by PAGE. Proteins are generally denatured by gel loading buffer prior to PAGE analysis. In the same way the beads and the captured protein can be boiled in gel loading buffer, the beads separated by a magnet and the eluted proteins analysed by PAGE. Alternatively, instead of PAGE and depending on the ELISA used, it may be possible to analyse the protein eluted in this way by ELISA as long as the sample is sufficiently diluted by the ELISA buffer used for capture. It may be helpful to supplement this buffer with 5% (w/v) BSA as a further precaution.

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