

Seprion™ Technology

Cancer Alzheimer's Parkinson's Huntington's Prions

PAD-Plates

ELISA-type detection of Protein Aggregation Diseases

Technical Manual

(96 Test kit)

For research use only



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I. Introduction

Microsens's unique and proprietary ligand Seprion™, can distinguish between abnormal aggregated protein and normal unaggregated protein. The ligand is provided coated onto microplates (**PAD-Plates**) for an ELISA-type assay. **PAD-Plates** have received USDA and EU approval for animal testing for prion disease (the Idexx Laboratories HerdCheck Test).

This ability to easily and efficiently separate the abnormal protein from normal protein facilitates research on Protein Aggregation Diseases. Research applications include the study and monitoring of Protein Aggregation Diseases in human and animal samples and drug screening *in vitro* and *in vivo*. To date **PAD-Plates** have been demonstrated to work with the following diseases, tissues and proteins:

Disease type	Tissue-type	Abnormal protein detected
Cancers and tumours	Multiple tissue types. Cell cultures	p53
Alzheimer's Disease	Brain from human and animal models. Cell cultures	β -amyloid and tau
Parkinson's Disease	Human brain	α -synuclein
Huntington's Disease	Animal models: brain, muscle, liver, kidney	huntingtin
Prion disease: Mad Cow Disease, (v)CJD, scrapie and CWD	Multiple tissue types from human and animal models. Cell cultures	prion protein

PAD-Plates are likely to work with other Protein Aggregation Diseases including: amylin in Diabetes Type II; crystalline in cataracts; antibody light chain, serum amyloid A, and β 2-microglobulin in amyloid including primary and secondary systemic amyloidosis; and superoxide dismutase 1 in amyotrophic lateral sclerosis but these diseases remain to be tested.

While the protocol for the detection using the kit is defined there are two Seprion-coated plate formats, SEP 1 and SEP 2 and two capture buffer formats, CB1 and CB2 which can be combined to give the best capture and thus the best signal for the particular aggregated protein to be investigated. For this reason, it is recommended that an evaluation kit, PADEval-01, containing both plate types and both buffer types should first be used in order to identify the best conditions for your protein of interest.

II. Kit contents for 96 tests

1x Seprion-coated microplate of 96 wells (SEP1 or SEP2)

1x Capture Buffer (CB1 or CB2)

(Note: evaluation kits (PADEval-01) include both plate types and both buffer types)

Store all reagents at 4-8°C

Allow plate and reagents to come to RT prior to opening and using them.

Reagents required but not supplied:

- Wash buffer. PBS containing 0.1% (v/v) Tween20
- Antibody diluent. The recommended antibody diluent is: PBS containing 4% (w/v) BSA, 1% (w/v) milk powder, 0.1% (v/v) Tween20 and 0.15MNaCl
- Detection antibody for the protein of interest. HRP conjugated antibodies are recommended. If the primary antibody is not conjugated a secondary conjugated antibody will be required. For example, if the primary antibody is a monoclonal, a HRP conjugated rabbit anti-mouse secondary antibody could be used.
- Substrate
- Stop solution.

III. Guidelines to sample preparation prior to using PAD-Plates.

Liquid samples

Liquid samples such as plasma, serum and CSF can be used. The sample volume should be limited to 100µl per test. Prior to use it may be beneficial to remove fibrinogen by heating at 56°C for 15 min followed by centrifugation at 20,000 x g for 5 min. The supernatant can then be used. Although many samples can be used directly, it may be useful to use protease treatments to reduce any effect of the sample matrix on the capture. Trypsin or proteinase K can be included in the capture step at final concentrations of 50-250µg/ml. The effect of adding these proteases and the exact concentration to be used should be determined empirically as the effect of these reagents will vary with sample and disease types.

Solid tissue samples

Tissue samples such as brain should be first homogenised in water or 5-20% sucrose as a 10-25% homogenate. A ribolyser or hand held homogeniser may be used. No more than 25 mg of tissue should be used per plate well in a total volume of 100µl homogenate. It may be beneficial to add SDS to the homogenate to a final concentration of 0.1-1% (w/v) to help solubilise the tissue. As with liquid samples, many samples can be used directly but it may be useful to use protease treatment to reduce any effect of the sample matrix on the capture (see above).

Soft tissue samples such as white cell samples, spleen and cell culture samples

It may be useful to treat these samples with DNase 1 before PAD-Plate Detection. Samples can be treated with 1% (v/v) Triton X-100, 0.1mM MgCl₂, 100µg/ml DNase 1 for 30 min prior to use. No more than 100µl of treated sample should be used in the subsequent separation procedure. As with liquid samples, many samples can be used directly, but it may be useful to use protease treatment after DNase treatment to reduce any effect of the sample matrix on the capture.

Storage of samples prior to separation

All samples and tissues processed or unprocessed can be stored frozen until detection is performed. Prior to analysis defrost and mix the samples thoroughly.

IV. PAD-Plate General Detection Protocol

For each capture, start with 100µl of sample that has been processed as described above (see the guidelines in section III)

1. In a microfuge tube or similar add either 25µl CB1 or CB2 to 100µl of sample. Mix thoroughly.
2. Add 100µl of the sample to the well of a PAD-plate (SEP1 or SEP2) and incubate at room temperature (RT) for 60-240 minutes.
3. Wash the wells five times with Wash buffer PBS + 0.1% Tween20.
4. Add 100µl of protein-specific antibody diluted in antibody diluent (the exact concentration used should be determined empirically but 0.2-1µg/ml is a good starting point) and incubate at RT for 60 minutes.

If the antibody used is already conjugated proceed to step 7. If a secondary antibody conjugate is used, proceed to step 5.

5. Wash three times with Wash buffer PBS+0.1% Tween20.
6. Add 100µl of an appropriate secondary antibody conjugate diluted in antibody diluent (the exact concentration used should be determined empirically but 0.5-1µg/ml is a good starting point) and incubate at RT for 45 minutes.
7. Wash five times with Wash buffer PBS 0.1% Tween20.
8. Add 100µl of appropriate conjugate substrate to each well and incubate at RT in the dark for 30 minutes.
9. Add 100µl of stop solution and read at the relevant wavelength.

V. Selected References

1. K. Sathasivam, A. Lane, J. Legleiter, A. Warley, B. Woodman, S. Finkbeiner, P. Paganetti, P. J. Muchowski, S. Wilson, and G. P. Bates. Identical oligomeric and fibrillar structures captured from the brains of R6/2 and knock-in mouse models of Huntington's disease. *Hum. Mol. Genet.* 2010 19(1): 65 - 78.
2. Lane A, Stanley CJ, Dealler S, and Wilson SM Polymeric Ligands with Specificity for Aggregated Prion Proteins. *Clin Chem.* 2003 49: 1774-1775.