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

Capture
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 25µl PAD Capture Buffer or Seprion Capture Buffer to the 100µl of sample. Mix thoroughly.
2. Add 100µl of the sample to a Seprion 1 or Seprion 2 capture plate and incubate at RT for 60-240 minutes.
3. Wash the wells five times with PBS 0.1% Tween20 (PBST).
4. Add 100µl of protein-specific antibody and incubate at RT for 60 minutes.
5. Wash three times with PBST.
6. Add 100µl of an appropriate secondary antibody conjugate and incubate at RT for 45 minutes.
7. Wash five times with PBST.
8. Add 100µl of 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.

Note: If the primary antibody is conjugated, then steps 5-6 can be omitted. Dilutions of antibodies used will have to be determined by the operator.

The recommended antibody diluent is: PBST, 4% (w/v) BSA, 1% (w/v) milk powder, 0.15 M NaCl.

V. References

I. Introduction

PAD-Plates are a unique tool that can be used to separate abnormal proteins present in many types of Protein Aggregation Diseases from the normal unaggregated protein (Lane et al. 2003, Clinical Chemistry 49:1774-1775). This ability to easily and efficiently separate the abnormal 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.

PAD-Plates are an ideal generic front-end separation technique that can be combined with a protein-specific antibody detection to enable the specific detection of abnormal aggregated proteins. To date, PAD-Plates have been demonstrated to work with β-amyloid, tau, α-synuclein, huntingtin, and prion proteins. Abnormally folded and aggregated proteins can be detected in vivo in human disease, in animal models, in cell culture, and in vitro using aggregated recombinant proteins. For example, PAD-Plates have been used to detect: abnormal β-amyloid and tau in Alzheimer’s Disease in both human disease and in animal models; and abnormal prion protein in both sporadic and new variant Creutzfeldt-Jakob Disease (CJD), in Bovine Spongiform Encephalopathy (BSE) in cows, in Chronic Wasting Disease (CWD) in deer and in scrapie, including atypical scrapie in sheep. A form of the Seprion-PAD technology used in PAD-Plates has received USDA and EU approval for animal testing for prion disease (the Idexx Herdchek Test).

*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 β2-microglobulin in amyloid including primary and secondary systemic amyloidosis; and superoxide dismutase 1 in amyotrophic lateral sclerosis but these diseases remain to be tested.

II. Kit contents for 100 separations

1 Seprion plate of 96 wells
1 Capture Buffer of 6 ml

Note: evaluation kits include two plate types and two 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:
PBS 0.1% Tween20
Antibody diluent (see below).

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

PAD-Plates are a flexible approach for the separation of abnormal proteins present in a range of Protein Aggregation Diseases from a diverse number of tissues and bodily fluids. While the protocol for the detection using the kit is defined there are two plate formats, Seprion 1 and Seprion 2 and two buffer formats, PAD Capture Buffer and Seprion Capture Buffer which can be combined to give the best signal for the aggregated protein to be investigated. For this reason, it is recommended that an evaluation kit containing both plate types and buffer types should first be used in order to identify the best conditions for your protein of interest.

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. 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. Again, as with liquid samples 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 (see above) it may be useful to use protease treatment to reduce any effect of the sample matrix on the capture.

Storage of samples prior to separation

Detection antibodies.
Conjugate Substrate
Stop Solution.