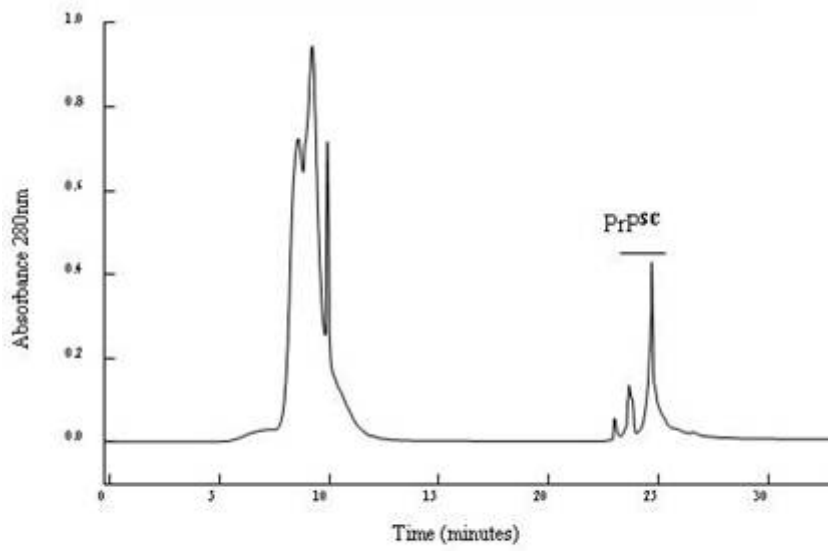
Assay of Pathogenic Prion Proteins

The pathogenic form of prions is associated with such diseases as **scrapie** in sheep, **Mad Cow Disease** (BSE) in cattle, **Creutzfeldt-Jakob Disease** (CJD) in humans, and **Chronic Wasting Disease** in elk and deer. It would be convenient to be able to detect these proteins in samples of **blood**, which is much easier to obtain than most other biological samples. PolyLC has developed such an assay in collaboration with Dr. Mary Jo Schmerr (Ames Laboratory, Iowa St. U.) (US Patent# 6,150,172). Starting with a 15-ml tube of blood, the buffy coat leucocytes are isolated and solubilized. Normal prion protein is destroyed with Proteinase K. The pathogenic prion can then be purified and isolated via HILIC on a column of our **PolyHYDROXYETHYL A™** material.

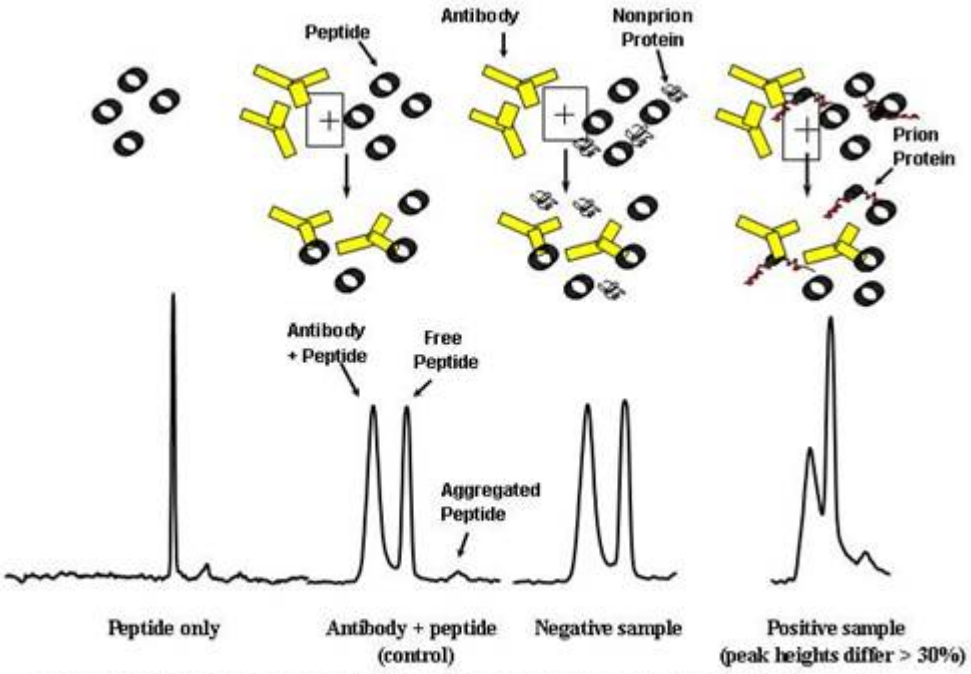


Column: PolyHYDROXYETHYL A
Gradient: Decreasing [ACN]

However, this can be accomplished much more quickly and cheaply with a disposable cartridge of the same material. This is our item# SPEHY1203. These are available in standard closed-top luer format for use with a syringe as well as in packed syringe barrel form for use with a vacuum manifold. 96-well SPE plates are also available.

The assay is based on binding of a fluorescent prion-sequence peptide to a polyclonal anti-prion antibody. The free and bound forms of the fluorescent peptide are separated by CE or SEC. If a HILIC-purified extract containing pathogenic prion protein is added

to the mixture, it competes with the fluorescent peptide for binding to the antibody and displaces some of it to the free peptide peak. A disparity in the height of the two peaks > 30% is a positive result.



COMPETITIVE IMMUNOASSAY OF SCRAPIE PRION BY CE.
Elapsed time of each electropherogram: 4'.

The Limit of Detection (LOD) of this assay is 150 pg of pathogenic prion protein in a 15-ml blood sample. This is 2x more sensitive than is necessary to detect a preclinical case of scrapie. Preliminary indications are that this assay can detect a case of disease several years before clinical symptoms are evident. Thus, this assay can prospectively be used to cull a herd of diseased animals years before the onset of symptoms. It may even be possible to eliminate the disease from the herd entirely with systematic assay of the animals at several intervals.

Assay of human blood samples, cerebrospinal fluid, pooled samples (e.g., growth media; bovine plasma), and processed food products: Contact PolyLC to discuss.