

# BetaPrion<sup>®</sup> BSE EIA Test Kit

Cat.No. 0104000102

Version 2.0/2006

Test for *in vitro* purification and detection of BSE PrP<sup>res</sup>

Within the European Union, this test is approved as rapid test for the BSE testing program on *cattle* which is set up in accordance with Regulation (EC) No 999/2001.

The producer of the rapid tests must have a quality assurance system in place agreed by the Community reference laboratory, which ensures that the test performance does not change. The producer must provide the test protocol to the Community reference laboratory. Sampling tools and modifications to the rapid test or to the test protocol (including sampling) may only be made following advance notification to the Community Reference Laboratory (CRL) and provided that the Community reference laboratory finds that the modification does not reduce the sensitivity, specificity or reliability of the rapid test. That finding shall be communicated to the Commission and to the national reference laboratories (Based on Regulation (EC) No 1053/2003 amending Regulation (EC) No 999/2001).

## BetaPrion<sup>®</sup> Sampling

Bovine brain of the obex region must be used as starting material for the BetaPrion<sup>®</sup> BSE EIA Test Kit.

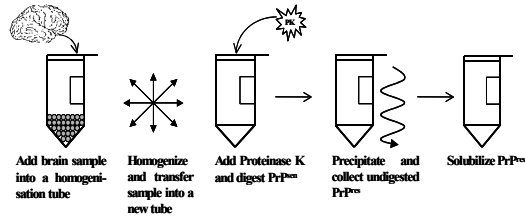
**Note:** After sample collection, a complete hemi-section of the brain stem with an intact obex region must remain available for confirmatory testing.

Sampling and laboratory testing must follow the Regulation (EC) No 999/2001 Chapter C which refers in terms of collection of samples to the latest edition of the "Manual of Standards for Diagnostic Tests and Vaccines of the International Office of Epizootic Diseases (OIE)) stating: "The preferred sample for immunoassay should be at, or as close to the obex as possible, but no further than 1.5 cm anterior to, the obex." The diagram shows that sampling area within the box.

Two opportunities for sampling:

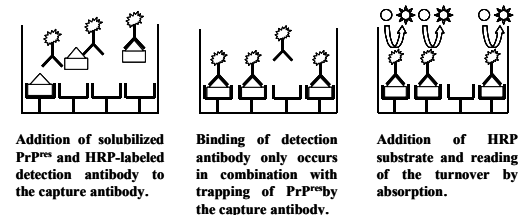
- (1) Cut 350 ± 50 mg nervous tissue from Obex with a scalpel and weigh the sample to ensure the correct amount.
- (2) Sample preparation using the AJ Roboscreen BSE sample syringe according to the instruction.

## BetaPrion<sup>®</sup> Purification



1. Homogenize 350 mg brain in a homogenisation tube for and homogenize for 45 sec using FastPrep24-System.
2. Incubation of 200 homogenate with 200 µl Proteinase K solution at 37°C for 15 min with continuous shaking.
3. Incubate with 400 µl precipitation solution at room temperature for 15 min and concentrate by centrifugation at 16000xg for 5 min.
4. Perform solubilisation using 25 µl solubilisation buffer and heating at 99°C for 5 min.

## BetaPrion<sup>®</sup> Detection



5. Mix the solubilized sample with 125 µl and controls with 500 µl of diluted HRP conjugate and vortex 2 sec, respectively.
6. Pipet 100 µl of each dilution per well and incubate at room temperature for 45 min.
7. Wash using the Columbus washer, program BSE-5.
8. Incubate 100 µl of staining solution and incubate in the dark at room temperature for 10 min.
9. Terminate reaction by adding 150 of stop solution and read the O.D. at 450 nm and 620 nm as reference wave length using the microplate reader.

## BetaPrion<sup>®</sup> Interpretation

The cut-off value is defined as 0.2 optical density OD<sub>450/620 nm</sub> and must be used for the discrimination of BSE positive samples from negative samples. The interpretation of data is done as follows:

- All samples with an OD<sub>450/620 nm</sub> below 0.2 are classified BSE negative.
- Samples with an OD<sub>450/620 nm</sub> ≥ 0.2 are classified initially reactive and must be re-tested in duplicate using the original homogenate. If one of the two duplicate readings has an OD<sub>450/620 nm</sub> ≥ 0.2 the sample is classified BSE positive and must be handled according to the respective national guidelines.

Samples and the corresponding tissue giving positive or inconclusive rapid test results should be sent to the NRL for confirmation.