

Instructions for Use

BetaPrion[®] BSE EIA Test Kit

Test for *in vitro* purification and detection of BSE PrP^{res}.



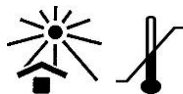
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Version 2020-3.6



2-10°C/ambient

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.



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Abbreviations:

| | |
|-------|-----------------------------------|
| BSE | Bovine spongiform encephalopathy |
| ELISA | Enzyme-linked immunosorbent assay |
| GLP | good laboratory praxis |
| PK | Proteinase K |
| HRP | Horseradish peroxidase |
| mab | monoclonal antibody |
| OD | optical density |
| RT | room temperature 22 ± 3 °C |
| TMB | Tetramethylbenzidine |



The BetaPrion® BSE EIA Test Kit consists of two modules, BetaPrion® BSE Purification Kit and BetaPrion® BSE Detection Kit.

BetaPrion® BSE Purification Kit allows the use of 2 ml tubes or 96 deep well plates.

One BetaPrion® BSE EIA Test Kit contains sufficient reagents to analyse 90 bovine brain samples.

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).

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1. Introduction

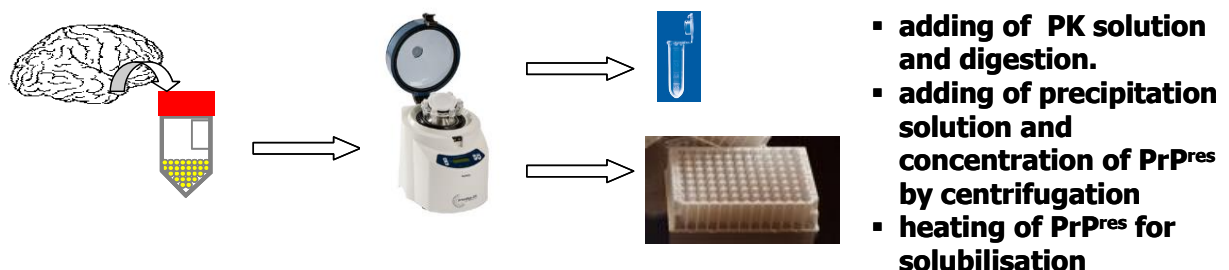
1.1. Brief general remarks

Prion diseases or transmissible spongiform encephalopathies are neuro-degenerative diseases that affect both humans and animals (Prusiner 1998). All prion diseases share the same molecular pathogenic mechanism that involves conversion of normal cellular prion protein (PrP^{sen}) into a form that is insoluble in non-ionic detergents and partially resistant to proteases (PrP^{res}) (Pan et al. 1993). Both PrP^{res} and PrP^{sen} are encoded within a single exon of a chromosomal gene coding for a protein of ~ 250 amino acids (Basler et al. 1986). Many mammalian PrPs have a 22 amino acid N-terminal signal sequence (Hope et al. 1986; Turk et al. 1988) and 23 amino acid C-terminal signal sequence encoding for attachment of a glycosylphosphatidylinositol anchor (Stahl et al. 1987, 1990). The mature protein of 209 amino acids contains one disulfide bond (Turk et al. 1988) and has two sites of asparagine-linked glycosylation (Endo et al. 1989; Oesch et al. 1995).

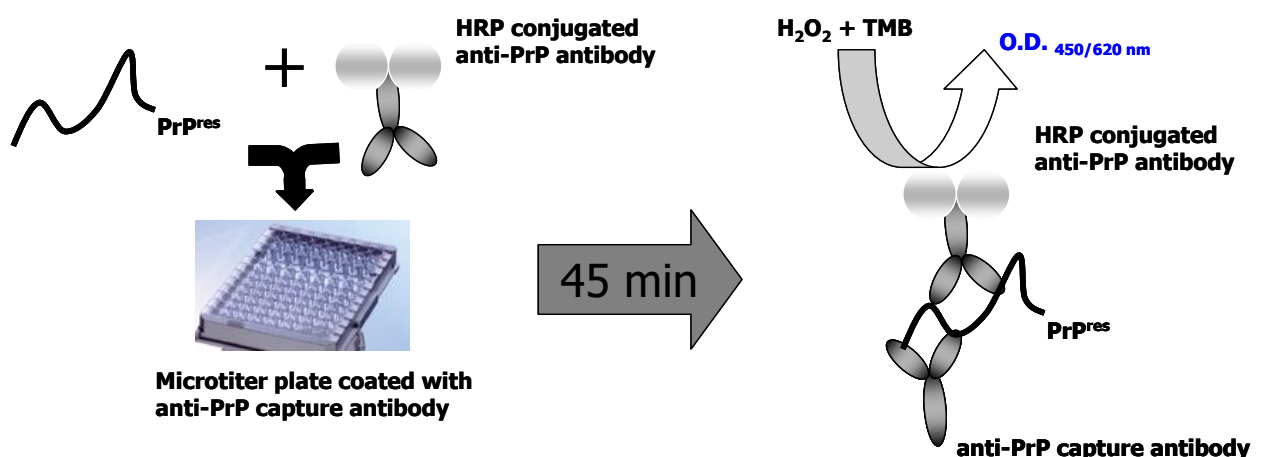
1.2. Short description of the test principle

The BetaPrion® BSE EIA Test Kit is a continuous 100 -110 min test for the detection of PrP^{res} in bovine brain samples. Specimens of bovine brain are homogenized and incubated with Proteinase K. Solubilized PrP^{res} is captured by a specific monoclonal anti-PrP antibody coated to the wells of a microtitre strip. Bound PrP^{res} is detected with a HRP-conjugated anti-PrP antibody. The wells are washed and a substrate solution is added. The developed colour indicates the existence of PrP^{res} in the specimen in comparison to a negative and a positive control in case the sample OD is greater than the declared cut-off.

1.2.1. Purification of PrP^{res}



1.2.2. Detection of PrP^{res}



2. Components not provided with the kit

For the Purification kit

- Homogenisation device, Ribolyzer, FastPrep®, FastPrep® 24 System (MP Biomedicals USA) or Precellys® 24 (Bertin France)
- BSE sample syringes
- 15 or 50 ml tubes, e.g. Falcon tubes, for the dilution of Proteinase K
- 2 ml tubes, e.g. Eppendorf (Germany)
- 96 ml deep well plates, e.g. Brand (Germany)
- Single and multi channel pipetting tools (adjustable, 100-1000 µl, 10-100 µl) and tips
- Desktop centrifuge (16.000g or 3.200 g), e.g. 5415D or 5815 (Eppendorf Germany)
- Thermostate, e.g. Thermomixer comfort (Eppendorf Germany) and heating block for 96 deep well plates, e.g. AJ Cybertron GmbH (Germany)
- 96 ml deep well plate aspiration tool, e.g. Washer (Kisker Germany)
- Syringe, e.g. 1ml and blunt end needles, 25Gx1.5" (e.g. Agesa Germany)
- Vortex mixer, e.g. Reax (VWR International Germany)
- Refrigerator 4-8°C

For the Detection Kit

- Single channel pipetting tools (adjustable, 100-1000 µl, 10-100 µl) and tips
- 15 ml tubes, e.g. Falcon tubes for the dilution of reagents
- Multichannel pipette (adjustable, 30-300 µl)
- Microplate reader CM Sunrise reader (Tecan Deutschland GmbH Germany)
- Microplate washer, e.g. CM Columbus washer (Tecan Deutschland GmbH Germany)

3. BetaPrion® BSE *Purification Kit*

3.1. Sampling

Bovine brain of the obex region (Figure 1) must be used as starting material for the BetaPrion® 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." Figure 1 shows the sampling area, either the left **or** the right hand side can be used.

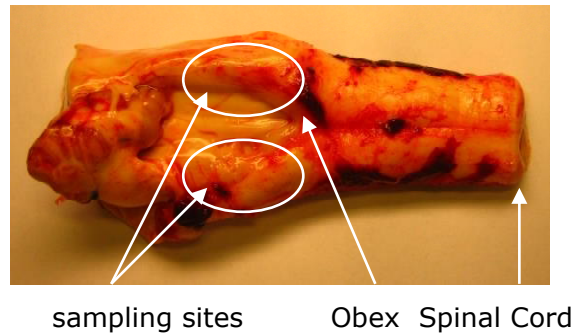



Figure 1: Sampling area

Two opportunities for sampling:

- (1) Cut 350 ± 50 mg nervous tissue from this area from the left **or** the right side of the brainstem 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 under (10) on page 14.

3.2. Kit components

| Short name | Description | Quantity  |
|-----------------------------|---|--|
| Homogenisation tubes | Transparent tubes (2 ml) with red cap containing Homogenisation buffer (1.3 ml) and ceramic beads, ready to use. Store at RT. | 96 tubes |
| P1 | Proteinase K buffer in transparent bottle with red label. Store at RT. | 1 x bottle (24 ml) |
| P2 | Proteinase K solution in transparent tube with yellow cap. Store at 4-8°C. | 1 x tube (0.6 ml) |
| P3 | Precipitation solution in brown bottle with red label containing detergents, ready to use. Store at RT. | 1 x bottle (45 ml) |
| P4 | Solubilisation buffer in transparent bottle with red label containing detergent solution, ready to use. Store at RT. | 1 x bottle (4 ml) |

3.3. Preparation of the Reagents

Ready to use reagents:

The homogenisation tubes, precipitation solution (P3) and the solubilisation buffer (P4) are ready to use reagents.

Reconstituted reagents:

Proteinase K buffer (P1) is required for the dilution of Proteinase K (P2) and must be used as follows:

| Number of samples | 2 ml tube purification | | 96 deep well plate purification | |
|-------------------|------------------------|------------------|---------------------------------|------------------|
| | P1 buffer (ml) | PK solution (μl) | P1 buffer (ml) | PK solution (μl) |
| 8 | 2.0 ± 0.20 | 50.0 ± 5.00 | 1.0 ± 0.10 | 50.0 ± 5.00 |
| 40 | 10.0 ± 1.00 | 250.0 ± 25.00 | 5.0 ± 0.50 | 250.0 ± 25.00 |
| 60 | 15.0 ± 1.50 | 375.0 ± 37.50 | 7.5 ± 0.75 | 375.0 ± 37.50 |
| 90 | 20.0 ± 2.00 | 500.0 ± 50.00 | 10.0 ± 1.00 | 500.0 ± 50.00 |

3.4. Test Procedure

Place the brain sample (350 ± 50 mg) into a homogenisation tube and homogenize for 45 sec at maximum speed (6.5) in FastPrep® or at 5500-5600 rpm in Precellys®24, respectively. Homogenized brain samples can be stored at -20 ± 2 °C for up to 12 months. For further use, thaw the samples at RT and vortex for 2 sec at maximal speed. Samples can be submitted to a maximum of 3 freezing-thawing cycles.

| 2 ml tube purification | 96 deep well plate purification |
|--|--|
| Transfer 200 ± 20 μl of homogenized sample with a 1-ml syringe using a fine needle (blunt end needle 25Gx1.5'') to a new 2 ml Eppendorf tube. | Transfer 300 ± 30 μl of homogenized sample with a 1-ml syringe using a fine needle (blunt end needle 25Gx1.5'') to 90 well of the 96 deep well plate. |
| Add 200 ± 20 μl of the reconstituted Proteinase K solution, mix by vortexing (2 sec at maximum speed) or inverting the tubes 3-6 times. | Add 100 ± 10 μl of the reconstituted Proteinase K solution, mix by pipetting or inverting the sealed plate 3 times. |
| Incubate at 37 ± 1 °C for 15 ± 1.5 min with continuous shaking (750 rpm). | Incubate at 37 ± 1 °C for 15 ± 1.5 min with continuous shaking (300 rpm). |
| Add 400 ± 40 μl precipitation solution, mix by vortexing (2 sec at maximal speed) or inverting the tubes 3-6 times and incubate the sample for 15 ± 1.5 min at RT. | Add 400 ± 40 μl precipitation solution, mix by pipetting or inverting the sealed plate 3 times and incubate the sample for 15 ± 1.5 min at RT. |
| Centrifuge 16.000 ± 1.600 g for 5 ± 0.5 min. Discard the supernatant carefully and removed the remaining supernatant by tipping the tubes on a paper towel. | Centrifuge 3.200 ± 320 g for 15 ± 1.5 min. Discard the supernatant carefully and removed the remaining supernatant by tipping the plate on a paper towel or aspirate the supernatant by pipetting tools. |
| Add 25 ± 2.5 μl solubilisation buffer to the pellet and heat at 99 ± 1 °C for 7 ± 0.5 min with intervall shaking (1200-1500 rpm). Cool down the tubes for at least 5 min at RT. Samples can be stored up to 6 h at 4-8°C or at RT. | Add 25 ± 2.5 μl solubilisation buffer to the pellet and heat at 99 ± 1 °C for 7 ± 0.5 min with intervall shaking (1200-1500 rpm). Cool down the plate for at least 5 min at RT. Samples can be stored up to 6 h at RT. |

3.5. Storage conditions and shelf life of the components

Store all components exceptional Proteinase K at room temperature (15-25°C). The tube containing Proteinase K has to be stored at 4-8°C. The guaranteed shelf life of the reagents is 12 months. The


reconstituted Proteinase K solution is stable up to 3 hours at room temperature (15-25 °C) or up to 8 hours at 4-8 °C.



Analyze the sample for PrP^{res} protein using the BetaPrion[®] BSE Detection Kit.

4. BetaPrion[®] BSE Detection Kit

4.1. Kit components

| Short name | Description | Quantity  |
|------------|---|--|
| D1 | Immunostrips coated with monoclonal anti-PrP capture antibody, stabilized, ready-to-use. | 1 immunoplate (12 x 8 wells) |
| D2 | Washing buffer (10x concentrate) in a transparent bottle, containing 0.01 % Na-Merthiolat as preservative. | 1 x bottle (100 ml) |
| D3 | Positive control in transparent tubes (2 ml) with blue caps containing lyophilized recombinant bovine prion protein. | 2 x tubes |
| D4 | Negative control in transparent tubes with white caps, ready-to-use. | 3 x tubes (3 x 0.1 ml) |
| D5 | Horseradish peroxidase conjugate in brown bottle with brown cap containing 8x concentrate of conjugated anti-PrP antibody. Preservative: 0.1 % Proclin 300. | 1 x bottle (2.5 ml) |
| D6 | Dilution buffer in transparent bottle containing buffered saline solution with BSA, ready to use. Preservative: 0.1 % Proclin 300. | 1 x bottle (20 ml) |
| D7 | Tetramethylbenzidine solution in brown tube with red cap containing a liquid stabilized TMB solution. | 1 x tube (1 ml) |
| D8 | Peroxide substrate solution in brown tube with green cap containing peroxide solution. | 1 x tube (1 ml) |
| D9 | Staining buffer in transparent bottle containing buffered saline solution. | 1 x bottle (20 ml) |
| D10 | Stop solution in transparent containing 1 M sulphuric acid, ready-to-use. | 1 x bottle (25 ml) |
| | Sealing tape | 1 x tape |

4.2. Preparation of the reagents

Allow immunostrips, washing buffer, dilution buffer, staining buffer and stop solution to equilibrate to room temperature prior performing the assay.

Ready to use reagents:

After prewarming to room temperature of the immunostrips (D1), open the bag with a scissors and remove the needed strips. Dilution buffer (D6) and Stop solution (D10) are ready to use reagents.

Reconstituted reagents:

Washing solution (D2):

Dilute the 10 x concentrated wash buffer with distilled water (add 900 ml of aqua dest to the 100 ml concentrate) before the washing step of the immunoassay.

Positive control (D3):

Add 0.5 ml of diluted HRP conjugate to positive control tube and vortex for 2 sec at the beginning of the immunoassay.

Negative control (D4) :

Add 0.5 ml of diluted HRP conjugate to negative control tube and vortex for 2 sec at the beginning of the immunoassay.

HRP conjugate (D5) :

Dilute the 8x HRP conjugate with dilution buffer (for 1 plate add 14 ml dilution buffer to 2 ml of conjugate) for diluted HRP conjugate.

Staining solution (D7 + D8 + D9)

Mix 12 ml of staining buffer (D9) with 300 µl of TMB solution (D7) and 180 µl peroxide substrate solution (D8) during the washing step of the immunoassay.

4.3. Immunoassay

Assay procedure for using 1 immunoplate (12x8 strips). If you use only individual strips please recalculate the reagent volumes required accordingly. For pipetting into the wells of the immunoplate please follow the pipetting scheme (chapter 7)

1. Add 125 ± 12.5 µl of diluted HRP conjugate to the solubilized sample resulted from the BetaPrion® BSE Purification Kit step 9 and mix it by 3 times pipetting or inverting or vortexing for 2 sec.
2. Pipet $4 \times 100 \pm 10$ µl of one negative and $2 \times 100 \pm 10$ µl of one positive control, respectively.
3. Pipet 100 ± 10 µl of the diluted sample per well of the coated immunostrips.
4. Cover the strips with sealing tape and incubate for 45 ± 4.5 min at RT.
5. Remove the sealing tape and wash the strips 5 times with $200-300 \pm 20-30$ µl diluted wash buffer either manually or by using the Columbus washer program BSE-5*.
6. Prepare the staining solution.
7. Dispense 100 ± 10 µl of prepared staining solution per well.
8. Incubate for 10 ± 1 min in the dark at room temperature (for this step do not seal the plate with the sealing tape).
9. Terminate reaction by adding 150 ± 15 µl of stop solution per well.
10. Read the O.D. at 450 nm and 620 nm as reference wave length using the microplate reader within 15 minutes after termination of the reaction.

*) Columbus washer program BSE-5 is a plate washing program using the overflow modus with 5×1000 µl washing buffer and an aspiration cycle at the end of the program.

4.4. Storage conditions and shelf life of reagents

Reconstituted reagents have a shelf life as follows:

| Short name | Reagent | Shelf life |
|------------|---------|------------|
| | | |

| | | |
|-----------|---|-------------------------|
| D1 | Coated immunostrips after opening of the bag. | 1 week at 4-8°C |
| D2 | 1X washing solution. | 1 week at RT |
| D5 | Diluted HRP conjugate | 4 h at 4-8°C |
| D8 | Mixed staining solution. | 2 h at RT in dark vials |

Store all reagents of the Detection kit at 4-8°C. The guaranteed shelf life of the reagents is 12 months.

5. Interpretation of the results

The cut-off value is defined as 0.2 optical density OD_{450/620 nm} 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_{450/620 nm} below 0.2 are classified BSE negative.
- Samples with an OD_{450/620 nm} > 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_{450/620 nm} > 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.

Validation of the test

The OD_{450/620 nm} value of the positive control must be at least 1.0 and of the OD_{450/620 nm} value of the negative control must be below 0.1. The plate must be repeated, when two of the negative controls have an OD_{450/620 nm} > 0.1.

6. Precautions

Prions are a unique class of pathogens which exhibits unusual resistance to conventional chemical and physical decontamination methods. A Biosafety level 3 laboratory is necessary for testing of BSE material. National legal regulations have to be considered. The quality of results generated with BetaPrion® BSE EIA Test Kit depends on compliance with the good Laboratory Practices (GLP).

Use disposable consumable supplies whenever possible. For decontamination of BSE positive material soak instruments in 2.5% sodium hypochlorite or 1 M NaOH for at least 1 hour or clean contaminated surfaces with those solutions. Liquids and waste should be decontaminated by autoclaving at 134°C for 1 hour.

7. Appendices

Pipetting scheme for the coated Microstrips

| | | | | | | | | | | | |
|---|---|----|----|----|----|----|----|----|----|----|----|
| N | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 | 75 | 83 |
| N | 4 | 12 | 20 | 28 | 36 | 44 | 52 | 60 | 68 | 76 | 84 |
| N | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 | 77 | 85 |

| | | | | | | | | | | | |
|---|----|----|----|----|----|----|----|----|----|----|----|
| N | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 | 78 | 86 |
| P | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 | 79 | 87 |
| P | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 | 88 |
| 1 | 9 | 17 | 25 | 33 | 41 | 49 | 57 | 65 | 73 | 81 | 89 |
| 2 | 10 | 18 | 26 | 34 | 42 | 50 | 58 | 66 | 74 | 82 | 90 |

Distribution of the controls and samples:

N = Negative control

P = Positive control

1-90 = Samples

How to contact **aj** ROBOSCREEN

If you are using the Internet access our homepage at www.roboscreen.com.

Please contact us by:

- E-mail: info@roboscreen.com,
- Phone: +49-341-9897340,
- Fax: +49-341-989734199.

8. Warranty notice/Additional general remarks

During the warranty period the BetaPrion® BSE EIA Test Kit allows precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.

Safety Statements

- **Homogenisation buffer** must not be classified according to the Directive 1272/2008/EC.
- **Buffer P1** contains Sodium Dodecylsulfate, which is eye Irritation according to the Directive 1272/2008/EC. Hazard (H) and precautionary statements (P) are listed below:

H302: = Harmful if swallowed.
H315: = Causes skin irritation.
H318: = Causes serious eye damage.
H319: = Causes serious eye irritation
H332: = Harmful if inhaled.
H335: = May cause respiratory irritation.
P262: = Do not get in eyes, on skin, or on clothing.
P280: = Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338: = IF IN EYES: Rinse cautiously with water for several minutes.
Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313: = If eye irritation persists: Get medical advice/attention.
Symbols according to EC No. 1272/2008 (CLP):



- **Buffer P2** contains proteinase K, which is Respiratory sensitizer according to the Directive 1272/2008/EC. Hazard (H) and precautionary statements (P) are listed below:

H315: = Causes skin irritation.
H319: = Causes serious eye irritation.
H334: = May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335: = May cause respiratory irritation.
P260: = Do not breathe dust/fume/gas/mist/vapours/spray.
P262: = Do not get in eyes, on skin, or on clothing.
P284: = [In case of inadequate ventilation] wear respiratory protection.
P304+P340: = IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P342+P311: = If experiencing respiratory symptoms: Call a POISON CENTER/doctor.

P501: = Dispose of contents/container to household waste.

Symbols according to EC No. 1272/2008 (CLP):



- **Buffer P3** contains Ethanol, which is a Flammable liquid, Poly(oxy-1,2-ethanediyl), α -[(1,1,3,3-tetramethylbutyl)phenyl]- ω -hydroxy, which is Acute Toxicity and Eye Damage and Organe, sweet, ext., which is a Acute aquatic toxicity - according to the Directive 1272/2008/EC. Hazard (H) and precautionary statements (P) are listed below:

H225: = Highly flammable liquid and vapour.

H226: = Flammable liquid and vapour.

H302: = Harmful if swallowed.

H304: = May be fatal if swallowed and enters airways.

H315: = Causes skin irritation.

H317: = May cause an allergic skin reaction.

H318: = Causes serious eye damage.

H400: = Very toxic to aquatic life.

H410: = Very toxic to aquatic life with long lasting effects.

H411: = Toxic to aquatic life with long lasting effects.

P210: = Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

P280: = Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338: IF IN EYES: = Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310: = Immediately call a POISON CENTER/doctor.

P273: = Avoid release to the environment.

P501: = Dispose of contents/container to household waste.

Symbols according to EC No. 1272/2008 (CLP):



- **Buffer P4** must not be classified according to the Directive 1272/2008/EC.
- **Component D1** must not be classified according to the Directive 1272/2008/EC.
- **Buffer D2** must not be classified according to the Directive 1272/2008/EC.
- **Component D3** contains 3(2H)-Isothiazolone, 5-chloro-2-methyl-, mixt. with 2-methyl-3(2H)-isothiazolone, which is skin sensitizer according to the Directive 1272/2008/EC. Hazard (H) and precautionary statements (P) are listed below:
 - H301: = Toxic if swallowed.
 - H311: = Toxic in contact with skin.
 - H314: = Causes severe skin burns and eye damage.
 - H317: = May cause an allergic skin reaction.
 - H331: = Toxic if inhaled.
 - H400: = Very toxic to aquatic life.
 - H410: = Very toxic to aquatic life with long lasting effects.
 - P280: = Wear protective gloves/protective clothing/eye protection/face protection.
 - P302+P352: = IF ON SKIN: Wash with plenty of water.
 - P333+P313: = If skin irritation or rash occurs: Get medical advice/attention.
 - P362+P364: = Take off contaminated clothing and wash it before reuse.
 - P501: = Dispose of contents/container to household waste

Symbols according to EC No. 1272/2008 (CLP):



- **Buffer D4** contains Poly(oxy-1,2-ethanediyl), α -[(1,1,3,3-tetramethylbutyl)phenyl]- ω -hydroxy, which is Eye Damage and Acute aquatic toxicity according to the Directive 1272/2008/EC. Hazard (H) and precautionary statements (P) are listed below:
 - H225: = Highly flammable liquid and vapour.
 - H226: = Flammable liquid and vapour.
 - H302: = Harmful if swallowed.
 - H304: = May be fatal if swallowed and enters airways.
 - H315: = Causes skin irritation.
 - H317: = May cause an allergic skin reaction.
 - H318: = Causes serious eye damage.
 - H400: = Very toxic to aquatic life.
 - H410: = Very toxic to aquatic life with long lasting effects.
 - H412: = Harmful to aquatic life with long lasting effects.
 - P280: = Wear protective gloves/protective clothing/eye protection/face protection.

- P305+P351+P338: = IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310: = Immediately call a POISON CENTER/doctor.
P273: = Avoid release to the environment.
P501: = Dispose of contents/container to household waste.

Symbols according to EC No. 1272/2008 (CLP):



- **Buffer D5** contains 3(2H)-Isothiazolone, 5-chloro-2-methyl-, mixt. with 2-methyl-3(2H)-isothiazolone, which is skin sensitizer according to the Directive 1272/2008/EC. Hazard (H) and precautionary statements (P) are listed below:
- H301: = Toxic if swallowed.
H311: = Toxic in contact with skin.
H314: = Causes severe skin burns and eye damage.
H317: = May cause an allergic skin reaction.
H331: = Toxic if inhaled.
H400: = Very toxic to aquatic life.
H410: = Very toxic to aquatic life with long lasting effects.
P280: = Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352: = IF ON SKIN: Wash with plenty of water.
P333+P313: = If skin irritation or rash occurs: Get medical advice/attention.
P362+P364: = Take off contaminated clothing and wash it before reuse.
P501: = Dispose of contents/container to household waste

Symbols according to EC No. 1272/2008 (CLP):



- **Buffer D6** contains 3(2H)-Isothiazolone, 5-chloro-2-methyl-, mixt. with 2-methyl-3(2H)-isothiazolone, which is skin sensitizer according to the Directive 1272/2008/EC. Hazard (H) and precautionary statements (P) are listed below:
- H301: = Toxic if swallowed.
H311: = Toxic in contact with skin.
H314: = Causes severe skin burns and eye damage.
H317: = May cause an allergic skin reaction.
H331: = Toxic if inhaled.
H400: = Very toxic to aquatic life.
H410: = Very toxic to aquatic life with long lasting effects.
P280: = Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352: = IF ON SKIN: Wash with plenty of water.
P333+P313: = If skin irritation or rash occurs: Get medical advice/attention.
P362+P364: = Take off contaminated clothing and wash it before reuse.
P501: = Dispose of contents/container to household waste

Symbols according to EC No. 1272/2008 (CLP):



- **Buffer D7** contains N, N-Dimethylacetamide, which is Reproductive toxicity, Acute Toxicity, eye Irritation and skin irritation according to the Directive 1272/2008/EC. Hazard (H) and precautionary statements (P) are listed below:
- H360D: = May damage the unborn child.
H261: = In contact with water releases flammable gases.
H332: = Harmful if inhaled.
H312: = Harmful in contact with skin.
H314: = Causes severe skin burns and eye damage.
H315: = Causes skin irritation.
H319: = Causes serious eye irritation.
P201: = Obtain special instructions before use.
P202: = Do not handle until all safety precautions have been read and understood.
P280: = Wear protective gloves/protective clothing/eye protection/face protection.
P263: = Avoid contact during pregnancy/while nursing.
P301+P312: = IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
P330: = Rinse mouth.
P305+P351+P338: = IF IN EYES: Rinse cautiously with water for several minutes.

| | |
|------------|---|
| | Remove contact lenses, if present and easy to do. Continue rinsing. |
| P337+P313: | = If eye irritation persists: Get medical advice/attention. |
| P302+P352: | = IF ON SKIN: Wash with plenty of water. |
| P332+P313: | = If skin irritation occurs: Get medical advice/attention. |
| P362+P364: | = Take off contaminated clothing and wash it before reuse. |
| P314: | = Get medical advice/attention if you feel unwell. |
| P405: | = Store locked up. |
| P501: | = Dispose of contents/container to special waste collection point |

Symbols according to EC No. 1272/2008 (CLP):



- **Buffer D8** must not be classified according to the Directive 1272/2008/EC.
- **Buffer D9** must not be classified according to the Directive 1272/2008/EC.
- **Buffer D10** contains Sulphuric acid as aqueous solution, which is skin irritation, eye Irritation and corrosive to metals according to the Directive 1272/2008/EC. Hazard (H) and precautionary statements (P) are listed below:

| | |
|-----------------|--|
| H290 | = May be corrosive to metals |
| H314: | = Causes severe skin burns and eye damage. |
| H315: | = Causes skin irritation. |
| H319: | = Causes serious eye irritation. |
| P234: | = Keep only in original container. |
| P280: | = Wear protective gloves/protective clothing/eye protection/face protection. |
| P264: | = Wash thoroughly after handling. |
| P305+P351+P338: | = IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
| P337+P313: | = If eye irritation persists: Get medical advice/attention. |
| P302+P352: | = IF ON SKIN: Wash with plenty of water. |
| P332+P313: | = If skin irritation occurs: Get medical advice/attention. |
| P362+P364: | = Take off contaminated clothing and wash it before reuse. |
| P406: | = Store in corrosive resistant / container with resistant inner liner. |

Symbols according to EC No. 1272/2008 (CLP):



9. General comments

All contents of the BetaPrion® BSE EIA Test Kit are produced under the guidelines of quality accordingly to the DIN EN ISO 13485 requirements.

10. Instruction for the sample preparation using AJ Roboscreen BSE sample syringe

Introduction

AJ Roboscreen offers a syringe for sampling of 350 ± 50 mg from the Obex region of the brainstem for testing with the BetaPrion® BSE EIA test kit.

It is recommended to use this instruction for taking the Obex sample with this syringe. Sampling using the syringe must be done by professional operators. The competence of the operators must be shown by a monitoring scheme in the lab that includes a professional training to know the anatomical orientation of the brainstem and the target area.

The syringe is easy to use. The syringe barrel is labelled with 0.1 ml steps (figure 2). Each 0.1 ml represents 100 mg tissue material. The correct amount of brain tissue to be sampled from the Obex is 350 ± 50 mg. Therefore, the operator must remove between 0.3 and 0.4 ml of tissue material.

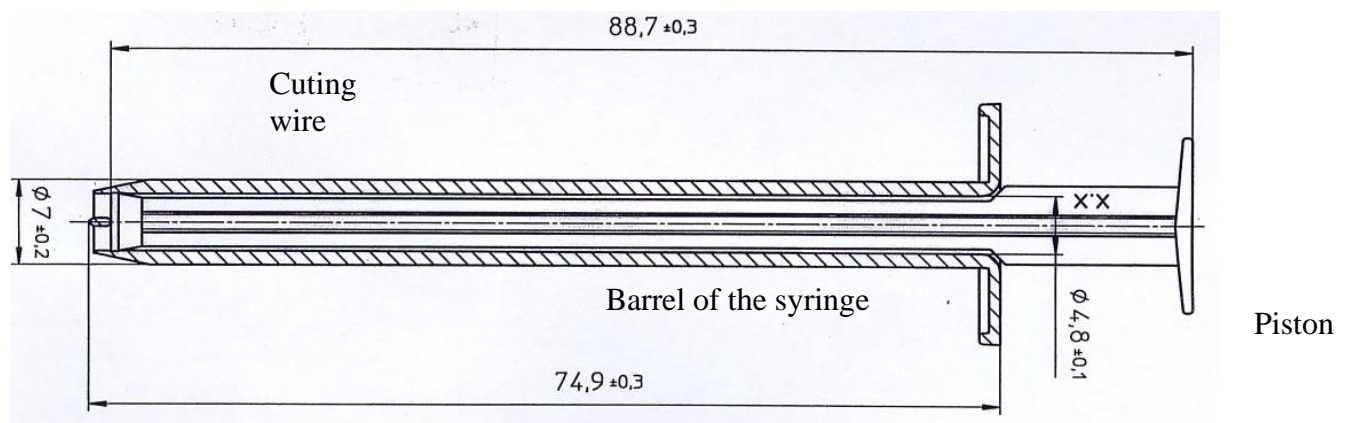


Figure 2: Scheme of the AJ Roboscreen BSE sample syringe

Histopathological description of the sample region

The preferred sample should be at, or within 1.5 cm anterior to, the Obex (figure 3).

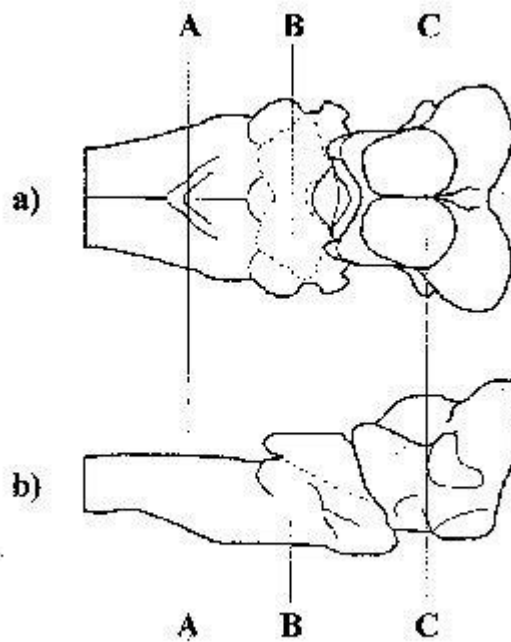


Figure 3: Brainstem after the removal of the Cerebellum, from a) dorsal, and b) lateral aspects.
Recommended levels at which sections for histopathological analysis should be taken (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals):
A-A = Medulla, at the Obex
B-B = Medulla through caudal cerebellar peduncles
C-C = midbrain through rostral colliculi

Sampling the rostral Medulla unilaterally for rapid testing must not compromise examination by histological or immunohistochemical means. The nucleus of the solitary tract (the target area in cattle) is small and lies close to midline (figure 4). The other early target area, the nucleus of the spinal tract of the trigeminal nerve, lies more laterally.

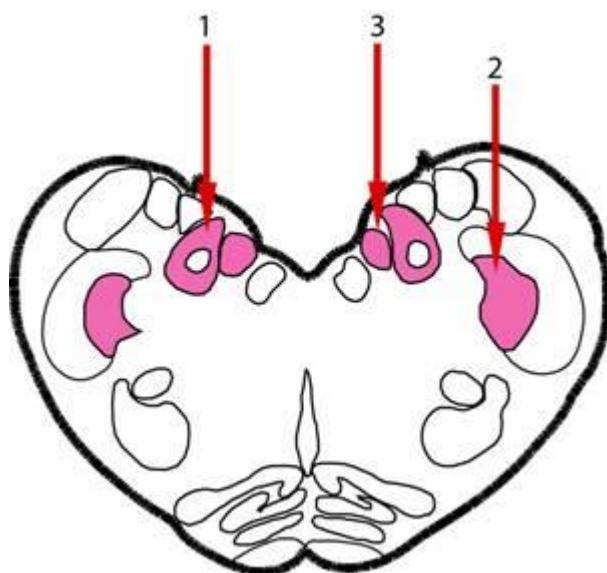


Figure 4: Cross section of the brain-stem at the level of the obex identifying the key target sites for diagnosis by histopathology and immunohistochemistry of BSE (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals):

- *nucleus of the solitary tract [1]*
- *nucleus of the spinal tract of the trigeminal nerve (V) [2]*
- *dorsal nucleus of the vagus [3]*

Usage of the syringe

The sampling region must be at or within 1.5 cm anterior to the Obex (figure 5).

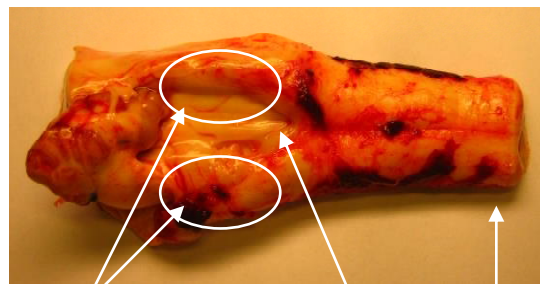


Figure 5: Sample area.

sampling sites

Obex

Spinal Cord

Before sampling, the length of the brain stem must be determined in order to be sure that the sampling is possible from the Obex with the syringe. In some brain tissue samples, the Spinal Cord is longer than the syringe. In this case cut the Spinal Cord with a scalpel, to enable the syringe to reach the sampling area.

Take the syringe with one hand and push the piston to the home position inside the syringe barrel. With the other hand take the brain stem and fix it on a disposable paper sheet. The caudal end of the brain stem/spinal cord should be accessible.

Now insert the syringe on the left or right side of the brain stem. One side of the brain stem must remain intact after sampling. This is reserved for confirmatory testing (figure 6). During insertion of the syringe into one side of the brain stem take care that it does not cross the midline.

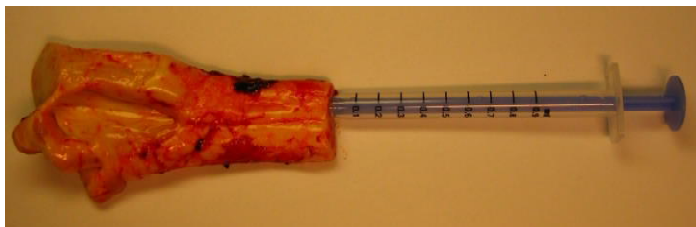


Figure 6: The syringe before the insertion into the right side of the brain stem.

Simultaneously, with inserting of the syringe into the tissue withdraw the piston from the syringe in order to generate a vacuum. This will allow the brain tissue to enter the barrel of the syringe. Press the sampling area with a finger of the other hand that fixes the brain. Stop the inserting if the end of the sampling area anterior to the Obex is reached (figure 7).



Figure 7: The syringe has reached the end of the sampling area.

Cut the sample by rotating the barrel two times. Slowly remove the syringe from the brain stem. The tissue sample should be remained inside the barrel.

Push the piston to remove any possible air gaps from the tip of the syringe. Be sure that all of sample is still inside the barrel. Push the piston to the next calibration on the syringe.

Take a homogenisation tube and remove the lid. Hold the syringe at the top of the tube.

Carefully push the piston to the middle of the label between 0.3- 0.4 ml and remove about 0.35 ml of tissue material. Cut the sample by gripping the top of the syringe against the top of the grinding tube.

Precautions

The sample syringes are disposable materials and must be used only once. The used syringe must be decontaminated and discarded as described below.

Autolysed samples could be taken by the syringe from the region that is identified as the Obex. For this case the sample should be raised by the syringe up to about 0.35 ml.

Damaged samples should be taken by a scalpel according to the BetaPrion manual.

Health and safety instructions

Wear disposable gloves when handling with reagents and samples from TSE risk materials.

Prions are a unique class of pathogens which exhibits unusual resistance to conventional chemical and physical decontamination methods. A Biosafety level 3** laboratory is necessary for testing of TSE material.

Use disposable consumable materials whenever possible. For decontamination of TSE positive material autoclaving at 134°C for 1 hour is recommended.

Surfaces, instruments and liquids that are not autoclaveable must be decontaminated in 2.5% sodium hypochlorite or 1 M NaOH for at least 1 hour.

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Incineration of all decontaminated waste is recommended.

The operator must receive specific training to work with risk material and prions. Decontamination of TSE risk materials and health and safety instructions must be comply with national legal regulations of the concerned country.

11. Symbols



Note.



Cat.-No.



No. of tests.



Store at:



Read instructions before use!



Use by:



Manufacturer



Keep away from heat or direct sun light.



Caution!

12. Notes

Contacts

Manufacturer/Distributor

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