TeSeE™ WESTERN BLOT

REAGENTS FOR IN VITRO CONFIRMATION OF SUSPECTED TSE POSITIVE SAMPLES

Validated and certified by the OIE for the purposes defined in this insert.
Registration number: 20090105

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Transmissible Spongiform Encephalopathies (TSE’s) were first reported in the eighteenth century in sheep (Scrapie) and more recently in cervids such as deer and elk (Chronic Wasting disease, CWD) and cattle (Bovine Spongiform Encephalopathy, BSE). Humans are also susceptible to certain forms of TSE such as Kuru, Creutzfeldt-Jakob Disease (CJD) or Gerstmann-Sträussler-Scheinker Syndrome (GSS). The emergence of new variant Creutzfeldt-Jakob Disease (vCJD) in the human population has been strongly linked to the dietary intake of BSE-infected meat or meat products. One of the main characteristics of TSEs is a progressive accumulation in the central nervous system of an abnormal isoform of natural or cellular prion protein (PrP\textsuperscript{c}), termed PrP\textsuperscript{res}. This disease specific PrP\textsuperscript{res} is characterised by an increased resistance to proteases. The TeSeE™ Western Blot assay permits qualitative identification of PrP\textsuperscript{res} after proteolytic treatment which results in a reduced molecular weight fragment due to ‘N’ terminus truncation.

Active/passive surveillance programs have been conducted worldwide to detect BSE, scrapie or CWD in infected animals. Those programs have resulted in the identification of increased numbers of positive cases at the screening laboratories. Those positive samples (suspected animals) are then systematically confirmed as “TSE-infected” by the demonstration of typical spongiform changes with histopathology, or with the detection of abnormal PrP by Immunohistochemistry (IHC), or of Scrapie Associated Fibrils (SAFs) by electron microscopy. These above confirmation techniques require technical expertise for the interpretation of the results and are time consuming and expensive. Western Blot technique can also be considered as an alternative method for confirmation of the TSE suspected samples.

The validation data for this kit have been certified by the OIE, based on expert review, as fit for the post-mortem detection of transmissible spongiform encephalopathies (TSEs) in cattle (bovine spongiform encephalopathy, BSE), in ovines and caprines (BSE and scrapie), and in cervids (Chronic Wasting Disease, CWD), and for the following purposes:

1. To confirm TSE suspected positive samples detected at the screening laboratories in countries with active/passive surveillance programmes. Any sample with a negative result according to the TeSeE™ Western Blot assay interpretation criteria, following a positive rapid test result, should be tested with one of the other OIE certified confirmatory methods, Immunohistochemistry (IHC) or SAF-Immunoblot;
2. To confirm the prevalence of infection with one of the TSE associated diseases (BSE, scrapie, CWD) in the context of an epidemiological survey in a low prevalence country;

3. To estimate prevalence of infection to facilitate risk analysis (e.g. surveys, implementation of disease control measures) and to assist the demonstration of the efficiency of eradication policies.

The TeSeE™ Western Blot assay is using the same assay principle as the Bio-Rad rapid assays (TeSeE™ SAP, TeSeE™ sheep/goat) that include the preliminary purification and concentration of the PrP\text{res}, associated to a highly sensitive immunoblotting. Then, it can be used efficiently for confirmation of any TSE suspected samples and for typing of TSE strains in sheep.

2 - ASSAY PRINCIPLE

The TeSeE™ Western Blot assay allows the detection of PrP\text{res} in nervous tissues (bovine, ovine, caprine, cervids, ...) or peripheral tissues (cervids) collected from infected animals.

The assay procedure begins with the digestion of cellular PrP protein (PrP\text{c}), followed by purification and concentration of disease specific PrP\text{res}. Detection of PrP\text{res} is carried out by electrophoretic migration then immunoblotting using a monoclonal antibody highly specific for PrP\text{res}.

The assay procedure includes the following steps:
- Sample homogenization,
- Digestion of PrP\text{c} with proteinase K,
- Purification and concentration of PrP\text{res},
- Electrophoresis and transfer onto a membrane,
- Immunoblotting.
3 - COMPOSITION OF THE KIT

<table>
<thead>
<tr>
<th>Labelling</th>
<th>Type of reagents</th>
<th>Presentation</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grinding Tubes</td>
<td>Grinding tubes containing ceramic beads in a buffer solution(^1)</td>
<td>1 bag (35 tubes)</td>
<td>+2°C to +25°C</td>
</tr>
<tr>
<td>A</td>
<td>Denaturing solution</td>
<td>1 vial (20 ml)</td>
<td>+2°C to +25°C</td>
</tr>
<tr>
<td>B</td>
<td>Clarifying solution Colouring: bromophenol blue Ready to use</td>
<td>1 vial (20 ml)</td>
<td>+2°C to +25°C</td>
</tr>
<tr>
<td>PK</td>
<td>Proteinase K Colouring: phenol red</td>
<td>1 vial (0.5 ml)</td>
<td>+2°C to +8°C</td>
</tr>
<tr>
<td>Ab I</td>
<td>Primary antibody(^1): anti-PrPmonoclonal antibody (10x)</td>
<td>1 vial (8 ml)</td>
<td>+2°C to +8°C</td>
</tr>
<tr>
<td>Ab II</td>
<td>Secondary antibody(^1): SheepAnti-Mouse IgG-(H+L)-HRP (10x)</td>
<td>1 vial (10 ml)</td>
<td>+2°C to +8°C</td>
</tr>
<tr>
<td>Bl</td>
<td>Blocking solution(^1) (10x)</td>
<td>1 vial (10 ml)</td>
<td>+2°C to +8°C</td>
</tr>
</tbody>
</table>

\(^1\) These reagents contain 0.1 % of ProClin™ 300 (preservative).

4 - SAMPLES

The TeSeE™ WESTERN BLOT assay is suitable for the detection of TSEs in cattle (Bovine Spongiform Encephalopathy, BSE), in ovine and caprine (BSE and scrapie), and in cervids (Chronic Wasting Disease, CWD). This test can be processed directly from the same sample homogenate (grinding tube) prepared for Bio-Rad rapid testing (TeSeE SAP, TeSeE sheep/goat).

**Bovine**: purification of PrPres is performed on samples from Central Nervous System (CNS). Since distribution of PrPres is heterogeneous in central nervous system, obex area from brainstem must be preferably sampled for optimal detection.

Sampling syringe (Ref.: 3551175) allows easy and rapid sampling of obex area in a secure way. Please refer to sampling protocol provided with sample syringes for detailed instructions on good sampling procedure.
Small ruminants: purification of PrP\textsuperscript{res} is performed on samples from Central Nervous System (CNS). Samples are cut and weighed individually.

Cervids: purification of PrP\textsuperscript{res} is performed on samples from Central Nervous System (CNS) or peripheral tissues (lymphoid nodes). Samples are cut and weighed individually.

5 - ASSAY PROCEDURE WITH MINI BLOT\textsuperscript{™} GEL

5.1 - ADDITIONAL REAGENTS AND MATERIAL REQUIRED

5.1.1 - REAGENTS AND DISPOSABLES

Graduated pipettes (5, 10, 25 ml), conical tubes (50 ml), 2 ml polypropylene micro-test tubes with caps. PARAFILM\textsuperscript{®}M Sealing films.

Sample purification

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laemmli sample buffer</td>
<td>30 ml</td>
<td>Bio-Rad, cat. Nr. 1610737</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>25 ml</td>
<td>Bio-Rad, cat. Nr. 1610710</td>
</tr>
<tr>
<td>SDS</td>
<td>100 g</td>
<td>Bio-Rad, cat. Nr. 1610301</td>
</tr>
<tr>
<td>Calibration syringes</td>
<td>200</td>
<td>Bio-Rad, cat. Nr. 3551174</td>
</tr>
</tbody>
</table>

Gel electrophoresis

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylamide 40% 29:1</td>
<td>500 ml</td>
<td>Bio-Rad, cat. Nr. 1610146</td>
</tr>
<tr>
<td>0.5 M Tris-HCl pH 6.8</td>
<td>1 L</td>
<td>Bio-Rad, cat. Nr. 1610799</td>
</tr>
<tr>
<td>1.5 M Tris-HCl pH 8.8</td>
<td>1 L</td>
<td>Bio-Rad, cat. Nr. 1610798</td>
</tr>
<tr>
<td>Bromophenol blue</td>
<td>10 g</td>
<td>Bio-Rad, cat. Nr. 1610404</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1 kg</td>
<td>Bio-Rad, Discontinued</td>
</tr>
<tr>
<td>Ammonium persulfate</td>
<td>10 g</td>
<td>Bio-Rad, cat. Nr. 1610700</td>
</tr>
<tr>
<td>TEMED</td>
<td>5 ml</td>
<td>Bio-Rad, cat. Nr. 1610800</td>
</tr>
<tr>
<td>Tris/Glycine/SDS (running buffer) (10x)</td>
<td>1 L</td>
<td>Bio-Rad, cat. Nr. 1610732</td>
</tr>
<tr>
<td>Kaleidoscope™ prestained standard</td>
<td>500 µl</td>
<td>Bio-Rad, cat. Nr. 1610375</td>
</tr>
<tr>
<td>MagicMark™ XP Western Standard (Molecular weight standard)</td>
<td>250 µl</td>
<td>Invitrogen, cat. Nr. LC5602</td>
</tr>
</tbody>
</table>

Immunoblotting

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (Normapur)</td>
<td>1L</td>
<td>VWR, cat. Nr. 20821-296</td>
</tr>
<tr>
<td>Tris/CAPS (transfer buffer) (10x)</td>
<td>1L</td>
<td>Bio-Rad, cat. Nr. 1610778</td>
</tr>
<tr>
<td>Filter paper</td>
<td>50 sheets</td>
<td>Bio-Rad, cat. Nr. 1703932</td>
</tr>
</tbody>
</table>

(transfer paper for Mini Blot™ handcast gels)
PVDF membrane (0.2 µm)  10 sheets  Bio-Rad, cat. Nr. 1620175
Tween® 20  100 ml  Bio-Rad, cat. Nr. 1706531
PBS (washing buffer) (10x)  1 L  Bio-Rad, cat. Nr. 1610780
ECL (substrate for conjugate)  125 ml  Amersham, cat. Nr. RPN2109
ECL Hyperfilms (18 x 24 cm)  25 films  Amersham, cat. Nr. RPN2103K
Development folders  30 folders  Applied Biosystems, cat Nr. T2258
Kodak developing solution LX24  to 20 L  VWR or Kodak
Kodak fixative solution AL4  to 20 L  VWR or Kodak

5.1.2 - EQUIPMENT

Adjustable pipettes (10, 40, 200, 1000 µl).
Graduated cylinder (1 L and 2 L), plastic forceps, trays, vortex.
Exposure cassette and red light for film development.

Sample purification

TeSeE™ Precess 48™  Bio-Rad, cat. Nr. 3590200
TeSeE™ Precess 24™  Bio-Rad, cat. Nr. 3591070
Block heater (3 blocks)  Bio-Rad, cat. Nr. 3589057
Heating block for block heater – 20 tubes  Bio-Rad, cat. Nr. 3589072
Centrifuge - 220/240 V  Bio-Rad, cat. Nr. 3591396
Drum rotor  Bio-Rad, cat. Nr. 3589189
Rotor adaptors - (x6)  Bio-Rad, cat. Nr. 3589191

Gel electrophoresis

Mini-PROTEAN® Tetra Cell, electrophoresis module  Bio-Rad, cat. Nr. 1658007
5 spacer plates  Bio-Rad, cat. Nr. 1653312
PowerPac™ HC power supply: 100/120 V - 220/240 V  Bio-Rad, cat. Nr. 1645052

Transfer

Trans-Blot® Cell  Bio-Rad, cat. Nr. 1703946

Immunoblotting

Western Processor base  Bio-Rad, Discontinued
Western Processor Mini Blot™ kit  Bio-Rad, Discontinued
5.2 - PREPARATION OF REAGENTS

5.2.1 - SAMPLE PURIFICATION

• **Proteinase K**
Solution of proteinase K diluted in reagent A:

- 1 ml Reagent A
- 20 µl Proteinase K

Mix well by inverting until you obtain a homogeneous solution. After reconstitution, diluted proteinase K is stable 10 hours at room temperature (+18°C to +30°C).

• **Laemmli solution**
Solution of SDS + 2-Mercaptoethanol + Laemmli sample buffer:

- 0.6 g SDS
- 1.5 ml 2-Mercaptoethanol

Mix by inverting.

- 28.5 ml Laemmli sample buffer

Solution is aliquoted into 4 ml aliquots and stored at -20°C. Thawed aliquots can be re-frozen.

**Note:** It is recommended to prepare Laemmli solution one hour before use allowing SDS to be completely dissolved.

5.2.2 - ELECTROPHORESIS

• **Hand cast discontinuous acrylamide gel**
The gel must be 1.5 mm thickness.
Using the Mini Blot™ casting module, the resolving gel (13.5% acrylamide, pH 8.8) is cast first, once the resolving gel is polymerized the stacking gel is added (3% acrylamide, pH 6.8).

Resolving gel (1 gel)

- 2.8 ml Acrylamide 40%, 29:1
- 1.7 ml 1.5 M Tris-HCl buffer, pH 8.8 / SDS (1)
- 1.3 ml 50% sucrose solution (2)
- 2.5 ml distilled water

Mix by inverting.

- 43 µl 10% Ammonium persulfate (3)
- 9 µl TEMED
Pour 7 ml of the gel solution into the plates and retain the residual solution as a control of polymerization. Gently overlay to the top with 1 ml of 0.3 M Tris-HCl pH 8.8 / SDS buffer (4) so that the gel surface doesn’t dry out. Let the gel polymerize for 15-20 minutes at room temperature (+18°C to +30°C). Check the residual solution is polymerized. Invert the plate assembly to eliminate excess of buffer.

Stacking gel (1 gel)

- 4 ml 3% Acrylamide solution (7)
- 28 µl 10% Ammonium persulfate (3)
- 6 µl TEMED

Mix by inverting.

Gently pour the stacking gel onto the resolving gel and retain the residual solution as a control of polymerization. Position the comb, taking care not to trap any bubble in the well positions.

Let the gel polymerize for 5-10 minutes at room temperature (+18°C to +30°C). Check the residual solution is polymerized.

(1) Solution of 1.5 M Tris-HCl buffer, pH 8.8 / SDS

- 0.2 g SDS
- 50 ml 1.5 M Tris-HCl buffer pH 8.8

Solution can be stored at +2°C to +8°C for 2 weeks.

(2) Solution of 50% Sucrose

- 25 g Sucrose
- to 50 ml Distilled water

Sucrose solution can be stored at +2°C to +8°C for 2 weeks.

(3) Solution of 10% Ammonium persulfate

- 5 g Ammonium persulfate
- to 50 ml Distilled water

Ammonium persulfate solution is aliquoted and stored at -20°C. Thawed solution can be stored at +2°C to +8°C for 2 weeks.

(4) Solution of 0.3 M Tris-HCl buffer, pH 8.8 / SDS

- 40 ml Distilled water
- 10 ml 1.5 M Tris-HCl buffer pH 8.8 / SDS

Solution can be stored at +2°C to +8°C for 2 weeks.
(5) Solution of 0.5 M Tris-HCl buffer, pH 6.8 / SDS
  ▶ 0.2 g SDS
  ▶ 50 ml 0.5 M Tris-HCl buffer pH 6.8
Solution can be stored at +2°C to +8°C for 2 weeks.

(6) Solution of 1% Bromophenol Blue
  ▶ 0.5 g Bromophenol Blue
  ▶ 50 ml Distilled water
Bromophenol Blue solution can be stored at room temperature (+18°C to +30°C) for 6 months.

(7) Solution of 3% acrylamide
  ▶ 3.8 ml Acrylamide 40%, 29:1
  ▶ 10 ml 0.5 M Tris-HCl buffer pH 6.8 / SDS (5)
  ▶ 6 ml Sucrose 50% (2)
  ▶ 500 µl Bromophenol Blue 1% (6)
  ▶ to 50 ml Distilled water
Solution can be stored at +2°C to +8°C for 2 weeks.

• Kaleidoscope™ prestained standard
The Kaleidoscope™ prestained standard is prepared during the sample denaturation before loading on the acrylamide gel.
Prepare a 1/12 dilution in Laemmli solution (for example 10 µl of the Kaleidoscope™ prestained standard + 110 µl of Laemmli solution).
Please refer to the Kaleidoscope™ prestained standard insert for storage conditions.

• MagicMark™ XP Western Standard
The MagicMark™ XP molecular weight is prepared during the sample denaturation before loading on the acrylamide gel.
Prepare a 1/12 dilution in Laemmli solution (for example 10 µl of MagicMark™ XP + 110 µl of Laemmli solution).
Please refer to MagicMark™ XP insert for storage conditions.

• Mini Blot™ migration buffer
Solution of Tris-Glycine-SDS (1x).
Prepare a 1/10 dilution. 1 L of diluted buffer is required for 1 tank:
  ▶ 900 ml Distilled water
  ▶ 100 ml Tris-Glycine-SDS buffer (10x)
Homogenize. Solution can not be stored.
5.2.3 - PROTEIN TRANSFER

• Transfer buffer
Solution of Tris/Caps-Ethanol 15%. 2.5 L is required for 1 transfer tank.
  ▶ 750 ml Distilled water
  ▶ 150 ml Pure ethanol
  ▶ 100 ml Tris/CAPS (10x)
Homogenize. Solution can not be stored.

5.2.4 - IMMUNOBLOTTING

• Wash solution 1
Solution of PBS (1x) + 0.1% Tween® 20. Approximately 500 ml is required for the complete process of 1 membrane.
  ▶ 900 ml Distilled water
  ▶ 100 ml PBS (10x)
  ▶ 1 ml Tween® 20
Thoroughly homogenize. Solution can be stored at +2°C to +8°C, overnight.

• Wash solution 2
Solution of PBS (1x). Approximately 100 ml is required for the complete process of 1 membrane.
  ▶ 900 ml Distilled water
  ▶ 100 ml PBS (10x)
Solution can be stored at room temperature (+18°C to +30°C) overnight.

• Blocking solution
During the transfer step, dilute the blocking solution (Bl) 1/10 in Wash solution 1. 20 ml of diluted blocking solution (1x) is required for 1 membrane.
  ▶ 18 ml Wash solution 1
  ▶ 2 ml Blocking solution (10x)
Homogenize by tube inverting.

• Diluted primary antibody
Just prior to use, dilute the primary antibody 1/10 in Wash solution 1. 15 ml of diluted primary antibody is required for 1 membrane.
  ▶ 13.5 ml Wash solution 1
  ▶ 1.5 ml Primary antibody (10x)
Homogenize by tube inverting.
• **Diluted secondary antibody (conjugate)**
  Just before use, dilute the secondary antibody 1/10 in Wash solution 1.  
  **20 ml of diluted conjugate is required for 1 membrane.**
  ▶ 18 ml  Wash solution 1  
  ▶ 2 ml  Secondary antibody (10x)
  Homogenize by tube inverting.

• **ECL**
  Substrate (ECL) must be prepared just before use.  
  **1 ml of substrate is required for 1 membrane.**
  ▶ 0.5 ml  Reagent 1  
  ▶ 0.5 ml  Reagent 2
  Homogenize the solution.

• **Development solution**
  ▶ 800 ml  Distilled water  
  ▶ 200 ml  Development product
  Solution can be stored at room temperature (+18°C to +30°C), in a darkroom for 15 days maximum.

• **Fixative solution**
  ▶ 800 ml  Distilled water  
  ▶ 200 ml  Fixative product
  Solution can be stored at room temperature (+18°C to +30°C), in a darkroom for 15 days maximum.
5.3 - SAMPLE PURIFICATION

The TeSeE™ WESTERN BLOT assay can be processed directly from the same sample homogenate (grinding tube) prepared for Bio-Rad rapid tests (TeSeE SAP, TeSeE sheep/goat).

**Sampling**

For peripheral tissues (lymph nodes) insert one medium bead (Ref.: 3551171) in the grinding tube, before to add the sample.

Take a mass of 350 mg ± 40 mg of nervous tissue (preferably in the obex area) or 200 mg ± 20 mg of peripheral tissue.

Deposit the sample in grinding tube, close firmly and proceed to the grinding step in the homogenizer (Ribolyser®, TeSeE™ PRECESS 48™ or TeSeE™ PRECESS 24™ - system).

**Sample grinding**

Place the tubes in the crown of the homogenizer.

Perform one agitation cycle with the following instrument parameters.

<table>
<thead>
<tr>
<th>Ribolyser®</th>
<th>TeSeE™ PRECESS 48™ or TeSeE™ PRECESS 24™</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nervous tissues</td>
</tr>
<tr>
<td>Time (sec.)</td>
<td>45</td>
</tr>
<tr>
<td>Speed</td>
<td>6.5</td>
</tr>
<tr>
<td>Program</td>
<td>-</td>
</tr>
</tbody>
</table>

When grinding is insufficient, another 1 or 2 agitation cycles can be performed\(^{(2)}\).

\(^{(1)}\) Wait a 5 minutes pause between the 2 agitation cycles.

**Sample calibration**

Remove the grinding tubes from the homogenizer, resuspend the homogenate by inverting before opening the tubes and aspirate 500 μl with the calibration syringe taking care to immerse the needle below the level of ceramic beads to avoid sampling tissue fragments.

Transfer each 500 μl sample into a 2 ml Eppendorf micro test-tube.

**Note:** At this stage, both grinding tubes after homogenisation and micro testtubes after sample calibration can be stored, closed:

- At room temperature (+18°C to +30°C) for 15 hours.
• At +2°C to +8°C for 72 hours.
• At -20°C for 1 year. Frozen samples must be thawed at room temperature (+18°C to +30°C).
Samples can be submitted to a maximum of 3 freezing/thawing cycles. Samples must always be homogenized by inverting before use.

**Proteinase K treatment**
Distribute 500 µl of reconstituted proteinase K solution (see paragraph 5.2.1) into each micro test-tube.
Homogenize the sealed tubes by inverting (10 times) and incubate at 37°C ± 2°C in a heating block incubator for 10 minutes.

**Precipitation of PrPres with reagent B**
Remove the tubes from the incubator. Open them and distribute 500 µl of reagent B into each tube. Homogenize by inverting until a homogeneous colour is obtained.

**Concentration of the PrPres by centrifugation**
Centrifuge the tubes for 7 minutes at 15000 g at 20°C.

**Sample clarifying**
Discard the supernatant by inverting over a waste container. Then dry the tubes by inverting onto absorbent paper for 5 minutes.
Distribute 100 µl of the Laemmli solution (see paragraph 5.2.1) into each micro test-tube.
Incubate for 5 minutes at room temperature (+18°C to +30°C).
Completely resolubilize the pellet by aspiration/dispensing with a pipette.
Incubate for 5 minutes at 100°C ± 5°C in a heating block incubator.
Remove the tubes from the incubator, homogenize by vortexing.
Centrifuge the tubes for 15 minutes at 15000 g at 20°C.
Transfer the supernatant to a new micro test-tube. Discard the tube containing the pellet.
At this stage, the supernatant can be stored frozen at -20°C for 24 hours; the samples must be thawed at room temperature (+18°C to +30°C) prior to use.
5.4 - ELECTROPHORESIS

The TeSeE™ Western Blot assay can be used for both confirmation of TSE suspected samples or for strain typing in sheep. The following procedure is applicable for confirmation of TSE suspected samples. Please contact your Bio-Rad representative for instruction protocol in case of strain typing application.

Gel preparation
Place the acrylamide gels (see paragraph 5.2.2) in the migration tank. Pour the migration buffer (see paragraph 5.2.2) into the electrophoresis tank on each side of the gels, up to the top of the wells. Carefully remove the combs and rinse each well with migration buffer, using a pipette.

Sample loading
Heat the samples for 4 minutes at 100 °C ± 5°C just prior to loading 15 µl/well. Load 15 µl of the diluted Kaleidoscope™ prestained standard and 15 µl of the diluted MagicMark™ XP (see paragraph 5.2.2).

Note: In case several gels are run at the same time, make sure that you stagger the loading of controls into different lanes for easy identification.

Differential migration of the samples
Run the gel at room temperature (+18°C to +30°C) for 90 minutes at 150 V. The blue line must be out of the gel.

5.5 - PROTEIN TRANSFER

The transfer buffer must be prepared before the end of the sample migration (see paragraph 5.2.3).

Protein transfer preparation
Cut the membrane to the gel dimensions. Always use forceps when handling the membrane.
Immerse the membrane in pure ethanol for 15 seconds, rinse in distilled water for 5 minutes, then for 10 minutes in the transfer buffer.
Carefully remove the gel from the glass plates and let it equilibrate for 10 minutes in the transfer buffer.
Gel sandwich preparation
Soak filter paper and fibre pads in the transfer buffer.
Open the transfer cassette, with transparent side on the left. Respectively place on the transparent side a fiber pad, a filter paper, the membrane* and the gel*.
Complete with a filter paper then a fibre pad and close the cassette.
Immerse it in the transfer tank previously filled to the indicated limit with transfer buffer.
*Remove any air bubbles which may have formed.
Note: In case several membranes are processed at the same time, label each membrane in the corner.

Transfer onto the PVDF membrane
Agitate during the transfer by using a magnetic stirring bar and run for 60 minutes at 115 V.

5.6 - IMMUNOBLOTTING

a) Upon completion of the protein transfer, open the blotting assembly and remove the membrane for development. Quickly immerse the membrane in Wash solution 2 (see paragraph 5.2.4), then place it in ethanol for 10 seconds before rinsing for 5 minutes in distilled water.
   Note: At this step, the membrane can be stored overnight in distilled water at +2°C to +8°C.
   Let the membrane adjust to room temperature (+18°C to +30°C) before to start the immunoblotting.

b) Eliminate distilled water and incubate the membrane for 30 minutes in blocking solution (see paragraph 5.2.4). Incubate under medium agitation.
   20 ml is sufficient for 1 membrane.
   Note: from this step until the step g), the Bio-Rad Western Processor can be used for agitation and washing steps (refer to instruction manual for settings).

c) Eliminate the blocking solution and incubate the membrane in diluted primary antibody (see paragraph 5.2.4) for 30 minutes at room temperature (+18°C to +30°C) under medium agitation.
   15 ml of diluted primary antibody is required for 1 membrane.
d) Eliminate the primary antibody solution and using Wash solution 1, briefly rinse the membrane, then wash twice for respectively 5 and 10 minutes, under fast agitation.

50 ml of Wash solution 1 is required for each cycle and for 1 membrane.

e) Eliminate Wash solution 1 and incubate the membrane for 20 minutes in diluted secondary antibody (see paragraph 5.2.4) at room temperature (+18°C to +30°C) under medium agitation.

20 ml of diluted secondary antibody is required for 1 membrane.

f) Eliminate the secondary antibody solution and using Wash solution 1, briefly rinse, then wash for respectively 5, 10 and 10 minutes under fast agitation.

50 ml of Wash solution 1 is required for each cycle and for 1 membrane.

g) Place the membrane in 50 ml of Wash solution 2 under slow agitation.

h) Drain the membrane on absorbent paper without blotting and place it in the plastic folder.

i) Add the ECL reagent (see paragraph 5.2.4). Eliminate the excess of reagent and air bubbles with absorbent paper. Place into the exposure cassette.

j) In a darkroom, cover the folder with a film and expose for 15 minutes. Film can be exposed longer or shorter time for optimal signal.

k) Immerse the film in developing solution for 45 seconds (see paragraph 5.2.4). Rinse in distilled water. Immerse the film in fixative solution until the film becomes completely transparent.

l) Wash with distilled water and let the film dry.
6 - ASSAY PROCEDURE WITH CRITERION™ XT GEL

6.1 - ADDITIONAL REAGENTS AND MATERIAL REQUIRED

6.1.1 - REAGENTS AND DISPOSABLES

Graduated pipettes (5, 10, 25 ml), conical tubes (50 ml), 2 ml polypropylene micro-test tubes with caps.
PARAFILM® M Sealing films.

Sample purification

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Supplier and Cat No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laemmli sample buffer</td>
<td>30 ml</td>
<td>Bio-Rad, cat. Nr. 1610737</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>25 ml</td>
<td>Bio-Rad, cat. Nr. 1610710</td>
</tr>
<tr>
<td>SDS</td>
<td>100 g</td>
<td>Bio-Rad, cat. Nr. 1610301</td>
</tr>
<tr>
<td>Calibration syringes</td>
<td>200</td>
<td>Bio-Rad, cat. Nr. 3551174</td>
</tr>
</tbody>
</table>

Gel electrophoresis

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Supplier and Cat No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criterion™ XT 12 % Bis-Tris 1 gel - 18 wells</td>
<td></td>
<td>Bio-Rad, cat. Nr. 3450118</td>
</tr>
<tr>
<td>XT-MOPS (running buffer) (20 x)</td>
<td>500 ml</td>
<td>Bio-Rad, cat. Nr. 1610788</td>
</tr>
<tr>
<td>Kaleidoscope™ prestained standard</td>
<td>500 µl</td>
<td>Bio-Rad, cat. Nr. 1610375</td>
</tr>
<tr>
<td>MagicMark™ XP Western Standard</td>
<td>250 µl</td>
<td>Invitrogen, cat. Nr. LC5602</td>
</tr>
</tbody>
</table>

(Molecular weight standard)

Immunobloting

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Supplier and Cat No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (Normapur)</td>
<td>1 L</td>
<td>VWR, cat. Nr. 20821-296</td>
</tr>
<tr>
<td>Tris/CAPS (transfer buffer) (10x)</td>
<td>1 L</td>
<td>Bio-Rad, cat. Nr. 1610778</td>
</tr>
<tr>
<td>Filter paper</td>
<td>50 sheets</td>
<td>Bio-Rad, cat. Nr. 1704085</td>
</tr>
<tr>
<td>(transfer paper for Criterion™ XT precast gels)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVDF membrane (0.2 µm)</td>
<td>10 sheets</td>
<td>Bio-Rad, cat. Nr. 1620175</td>
</tr>
<tr>
<td>Tween® 20</td>
<td>100 ml</td>
<td>Bio-Rad, cat. Nr. 1706531</td>
</tr>
<tr>
<td>PBS (washing buffer) (10x)</td>
<td>1 L</td>
<td>Bio-Rad, cat. Nr. 1610780</td>
</tr>
<tr>
<td>ECL (substrate for conjugate)</td>
<td>125 ml</td>
<td>Amersham, cat. Nr. RPN2109</td>
</tr>
<tr>
<td>ECL Hyperfilms (18 x 24 cm)</td>
<td>25 films</td>
<td>Amersham, cat. Nr. RPN2103K</td>
</tr>
<tr>
<td>Development folders</td>
<td>30 folders</td>
<td>Applied Biosystems, cat. Nr. T2258</td>
</tr>
<tr>
<td>Kodak developing solution LX24</td>
<td>to 20 L</td>
<td>VWR or Kodak</td>
</tr>
<tr>
<td>Kodak fixative solution AL4</td>
<td>to 20 L</td>
<td>VWR or Kodak</td>
</tr>
</tbody>
</table>
6.1.2 - EQUIPMENT

Adjustable pipettes (10, 40, 200, 1000 μl).
Graduated cylinder (1L and 2L).
Plastic forceps, trays, vortex.
Exposure cassette and red light for film development.

Sample purification

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplier / Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>TeSeE™ Precess 48™,</td>
<td>Bio-Rad, cat. Nr. 3590200</td>
</tr>
<tr>
<td>TeSeE™ Precess 24™,</td>
<td>Bio-Rad, cat. Nr. 3591070</td>
</tr>
<tr>
<td>Block heater (3 blocks)</td>
<td>Bio-Rad, cat. Nr. 3589057</td>
</tr>
<tr>
<td>Heating block for block heater – 20 tubes</td>
<td>Bio-Rad, cat. Nr. 3589072</td>
</tr>
<tr>
<td>Centrifuge - 220/240 V</td>
<td>Bio-Rad, cat. Nr. 3591396</td>
</tr>
<tr>
<td>Drum rotor</td>
<td>Bio-Rad, cat. Nr. 3589189</td>
</tr>
<tr>
<td>Rotor adaptors - (x6)</td>
<td>Bio-Rad, cat. Nr. 3589191</td>
</tr>
</tbody>
</table>

Gel electrophoresis

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplier / Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criterion™ XT Cell</td>
<td>Bio-Rad, cat. Nr. 1656001</td>
</tr>
<tr>
<td>PowerPac™ HC power supply: 100/120 V - 220/240 V</td>
<td>Bio-Rad, cat. Nr. 1645052</td>
</tr>
</tbody>
</table>

Transfer

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplier / Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criterion™ XT blotter</td>
<td>Bio-Rad, cat. Nr. 1704070</td>
</tr>
</tbody>
</table>

Immunoblotting

<table>
<thead>
<tr>
<th>Item</th>
<th>Supplier / Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Processor base</td>
<td>Bio-Rad, Discontinued</td>
</tr>
<tr>
<td>Western Processor Criterion™ XT kit</td>
<td>Bio-Rad, Discontinued</td>
</tr>
</tbody>
</table>
6.2 - PREPARATION OF REAGENTS

6.2.1 - SAMPLE PURIFICATION

• Proteinase K
  Solution of proteinase K diluted in reagent A:
  ▶ 1 ml Reagent A
  ▶ 20 µl Proteinase K
  Mix well by inverting until you obtain a homogeneous solution. After reconstitution, diluted proteinase K is stable 10 hours at room temperature (+18°C to +30°C).

• Laemmli solution
  Solution of SDS + 2-Mercaptoethanol + Laemmli sample buffer:
  ▶ 0.6 g SDS
  ▶ 1.5 ml 2-Mercaptoethanol
  Mix by inverting.
  ▶ 28.5 ml Laemmli sample buffer
  Solution is aliquoted into 4 ml aliquots and stored at -20°C. Thawed aliquots can be re-frozen.
  Note: It is recommended to prepare Laemmli solution one hour before use allowing SDS to be correctly dissolved.

6.2.2 - ELECTROPHORESIS

• Kaleidoscope™ prestained standard
  The Kaleidoscope™ prestained standard is prepared during the sample denaturation before loading on the acrylamide gel.
  Prepare a 1/12 dilution in Laemmli solution, for example 10 µl of the Kaleidoscope™ prestained standard + 110 µl of Laemmli solution.
  Please refer to the Kaleidoscope™ prestained standard insert for storage conditions.

• MagicMark™ XP Western Standard
  The MagicMark™ XP molecular weight is prepared during the sample denaturation before loading on the acrylamide gel.
  Prepare a 1/12 dilution in Laemmli solution, for example 10 µl of MagicMark™ XP + 110 µl of Laemmli solution.
  Please refer to MagicMark™ XP insert for storage conditions.
• **Criterion™ XT migration buffer**  
  Solution of MOPS (1x).  
  Prepare a 1/20 dilution. 1 L of diluted buffer is required for 1 tank:  
  ▶ 950 ml Distilled water  
  ▶ 50 ml MOPS buffer (20x)  
  Homogenize. Solution cannot be stored.

**6.2.3 - PROTEIN TRANSFER**

• **Transfer buffer**  
  Solution of Tris/CAPS-Ethanol 15%. **Approximately 2 L is required for 1 migration tank.**  
  ▶ 750 ml Distilled water  
  ▶ 150 ml Pure ethanol  
  ▶ 100 ml Tris/CAPS (10x)  
  Homogenize. Solution cannot be stored.

**6.2.4 - IMMUNOBLOTTING**

• **Wash solution 1**  
  Solution of PBS (1x) + 0.1% Tween® 20. **Approximately 1 L is required for the complete process of 1 membrane.**  
  ▶ 900 ml Distilled water  
  ▶ 100 ml PBS (10x)  
  ▶ 1 ml Tween® 20  
  Thorougly homogenize. Solution is stored at +2°C to +8°C, overnight.

• **Wash solution 2**  
  Solution of PBS (1x). **Approximately 200 ml is required for the complete process of 1 membrane.**  
  ▶ 900 ml Distilled water  
  ▶ 100 ml PBS (10x)  
  Solution is stored at room temperature (+18°C to +30°C) overnight.

• **Blocking solution**  
  During the transfer step, dilute the blocking solution (Bl) 1/10 in Wash solution 1. **40 ml of diluted blocking solution is required for 1 membrane.**  
  ▶ 36 ml Wash solution 1  
  ▶ 4 ml Blocking solution (10x)  
  Homogenize by tube inverting.
• **Diluted primary antibody**
  Just prior to use, dilute the primary antibody 1/10 in Wash solution 1.  
  **30 ml of diluted antibody is required for 1 membrane.**  
  ▶ 27 ml  Wash solution 1  
  ▶ 3 ml  Primary antibody (10x)  
  Homogenize by inverting.

• **Diluted secondary antibody (conjugate)**
  Just prior to use, dilute the secondary antibody 1/10 in Wash solution 1.  
  **40 ml of diluted conjugate is required for 1 membrane.**  
  ▶ 36 ml  Wash solution 1  
  ▶ 4 ml  Secondary antibody (10x)  
  Homogenize by tube inverting.

• **ECL**
  Substrate (ECL) must be prepared just prior to use.  
  **2 ml of substrate is required for 1 membrane.**  
  ▶ 1 ml  Reagent 1  
  ▶ 1 ml  Reagent 2  
  Homogenize.

• **Development solution**  
  ▶ 800 ml  Distilled water  
  ▶ 200 ml  Development product  
  Solution is stored at room temperature (+18°C to +30°C), in a darkroom for 15 days maximum.

• **Fixative solution**  
  ▶ 800 ml  Distilled water  
  ▶ 200 ml  Fixative product  
  Solution is stored at room temperature (+18°C to +30°C), in a darkroom for 15 days maximum.
6.3 - SAMPLE PURIFICATION

The TeSeE™ WESTERN BLOT assay can be processed directly from the same sample homogenate (grinding tube) prepared for Bio-Rad rapid tests (TeSeE™ SAP, TeSeE™ sheep/goat).

**Sampling**

For peripheral tissues (lymph nodes) insert one medium bead (Ref.: 3551171) in the grinding tube, before to add the sample. Take a mass of 350 mg ± 40 mg of nervous tissue (preferably in the obex area) or 200 mg ± 20 mg of peripheral tissue. Deposit the sample in grinding tube, close firmly and proceed to the grinding step in the homogenizer (Ribolyser®, TeSeE™ PRECESS 48™ or TeSeE™ PRECESS 24™ - system).

**Sample grinding**

Place the tubes in the crown of the homogenizer. Perform one agitation cycle with the following instrument parameters.

<table>
<thead>
<tr>
<th></th>
<th>Ribolyser®</th>
<th>TeSeE™ PRECESS 48™ or TeSeE™ PRECESS 24™</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nervous tissues</td>
<td>Peripheral tissues</td>
</tr>
<tr>
<td>Time (sec.)</td>
<td>45</td>
<td>2 x 45(^{(1)})</td>
</tr>
<tr>
<td>Speed</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Program</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

When grinding is insufficient, another 1 or 2 agitation cycles can be performed\(^{(2)}\).

\(^{(1)}\) Wait a 5 minutes pause between the 2 agitation cycles.

**Sample calibration**

Remove the grinding tubes from the homogenizer, resuspend the homogenate by inverting before opening the tubes and aspirate 500 μl with the calibration syringe taking care to immerse the needle below the level of ceramic beads to avoid sampling tissue fragments. Transfer each 500 μl sample into 2 ml Eppendorf micro test-tube.

**Note:** at this stage, both grinding tubes after homogenisation and micro test-tubes after sample calibration can be stored, closed:

- At room temperature (+18°C to +30°C) for 15 hours.
• At +2°C to +8°C for 72 hours.
• At -20°C for 1 year. Frozen samples must be thawed at room temperature (+18°C to +30°C).

Samples can be submitted to a maximum of 3 freezing/thawing cycles. Samples must always be homogenized by inverting before use.

Proteinase K Treatment
Distribute 500 µl of reconstituted proteinase K solution (see paragraph 6.2.1) into each micro test-tube.
Homogenize the sealed tubes by inverting (10 times) and incubate at 37°C ± 2°C in a heating block incubator for 10 minutes.

Precipitation of PrP<sub>res</sub> with reagent B
Remove the tubes from the incubator. Open and distribute 500 µl of reagent B into each tube. Homogenize by inverting until a homogeneous colour is obtained.

Concentration of the PrP<sub>res</sub> by centrifugation
Centrifuge the tubes for 7 minutes at 15000 g at 20°C.

Sample clarifying
Discard the supernatant by inverting over a waste container. Then dry the tubes by inverting onto absorbent paper for 5 minutes.
Distribute 100 µl of the Laemmli solution (see paragraph 6.2.1) into each micro test-tube.
Incubate for 5 minutes at room temperature (+18°C to +30°C).
Completely resolubilise the pellet by aspiration/dispensing with a pipette.
Incubate for 5 minutes at 100°C ± 5°C in a heating block incubator.
Remove the tubes from the incubator, homogenize by vortexing.
Centrifuge the tubes for 15 minutes at 15000 g at 20°C.
Transfer the supernatant to a new micro test-tube. Discard the tube containing the pellet.
At this stage, the supernatant can be stored frozen at -20°C for 24 hours; the samples must be thawed at room temperature (+18°C to +30°C) prior to use.
6.4 - ELECTROPHORESIS

The TeSeE™ Western Blot assay can be used for both confirmation of TSE suspected samples or for strain typing in sheep. The following procedure is applicable for confirmation of TSE suspected samples. Please contact your Bio-Rad representative for instruction protocol in case of strain typing application.

Gel preparation
Remove the plastic band on the bottom of the plastic plate and place the acrylamide gels (see paragraph 6.2.2) in the migration tank. Pour the migration buffer (see paragraph 6.2.2) on each side of the gel up to the top of the wells and into the electrophoresis tank. Carefully remove the combs and rinse each well with migration buffer, using a pipette.

Sample loading
Heat the samples for 4 minutes at 100 °C ± 5°C just prior to loading 15 µl/well. Load 15 µl of the diluted Kaleidoscope™ prestained standard and 15 µl of the diluted MagicMark™ XP (see paragraph 6.2.2).
Note: In case several gels are run at the same time, make sure that you stagger the loading of controls into different lanes for easy identification.
Differential migration of the samples
Run the gel at room temperature (+18°C to +30°C) for 50 minutes at 200 V.

6.5 - PROTEIN TRANSFER

The transfer buffer must be prepared before the end of the sample migration (see paragraph 6.2.3).

Protein transfer preparation
Cut the membrane to the gel dimensions. Always use forceps when handling the membrane.
Immerse the membrane in pure ethanol for 15 seconds, rinse in distilled water for 5 minutes, then for 10 minutes in the transfer buffer.
Carefully remove the gel from the plastic plates and let it equilibrate for 10 minutes in the transfer buffer.
**Gel sandwich preparation**
Soak filter paper and fibre pads in the transfer buffer.
Open the transfer cassette, with red side on the left. Respectively place on the red side a fiber pad, a filter paper, the membrane* and the gel*. Complete with a filter paper then a fibre pad and close the cassette.
Immerse it in the transfer tank, previously filled to the indicated limit with transfer buffer. A frozen ice pack is added prior to fill the tank.
*Remove any air bubbles which may have formed.
**Note:** In case several membranes are processed at the same time, label each membrane in the corner.

**Transfer onto the PVDF membrane**
Agitate during the transfer by using a magnetic stirring bar and run for 60 minutes at 115 V.

**6.6 - IMMUNOBLOTTING**

a) Upon completion of the protein transfer, open the blotting assembly and remove the membrane for development. Quickly immerse the membrane in Wash solution 2 (see paragraph 6.2.4), then place it in ethanol for 10 seconds before rinsing for 5 minutes in distilled water.
**Note:** At this step, the membrane can be stored overnight in distilled water at +2°C to +8°C.
Let the membrane adjust to room temperature (+18°C to +30°C) before to start the immunoblotting.

b) Eliminate distilled water and incubate the membrane for 30 minutes in blocking solution (see paragraph 6.2.4). Incubate under medium agitation.
**40 ml is required for 1 membrane.**
**Note:** from this step until step g), the Bio-Rad Western Processor can be used for agitation and washing steps (refer to instruction manual for settings).

c) Eliminate the blocking solution and incubate the membrane in diluted primary antibody (see paragraph 6.2.4) for 30 minutes at room temperature (+18°C to +30°C) under medium agitation.
**30 ml of diluted primary antibody is required for 1 membrane.**
d) Eliminate the primary antibody solution and using Wash solution 1, briefly rinse the membrane, then wash twice for respectively 5 and 10 minutes, under fast agitation. **100 ml of Wash solution 1 is required for each cycle and for 1 membrane.**

e) Eliminate the Wash solution 1 and incubate the membrane for 20 minutes in diluted secondary antibody (see paragraph 6.2.4) at room temperature (+18°C to +30°C) under medium agitation. **40 ml of diluted secondary antibody is required for 1 membrane.**

f) Eliminate the secondary antibody solution and using Wash solution 1, briefly rinse for respectively 5, 10 and 10 minutes under fast agitation. **100 ml of Wash solution 1 is required for each cycle and for 1 membrane.**

g) Place the membrane in 100 ml of Wash solution 2 under slow agitation.

h) Drain the membrane on absorbent paper without blotting and place it in the plastic folder.

i) Add the ECL reagent (see paragraph 6.2.4). Eliminate the excess of reagent and air bubbles with absorbent paper. Place into the exposure cassette.

j) In a darkroom, cover the folder with a film and expose for 15 minutes. Film can be exposed longer or shorter time for optimal signal.

k) Immerse the film in developing solution for 45 seconds (see paragraph 6.2.4). Rinse in distilled water. Immerse the film in fixative solution until the film becomes completely transparent.

l) Wash with distilled water and let the film dry.
7 - INTERPRETATION OF RESULTS

Figure 1 shows the expected band patterns for TSE negative samples, TSE positive samples in various animal species and molecular weight controls (positions 3 and 9).

![Figure 1](image)

**Negative samples** (positions 1 and 10) were treated with proteinase K. They do not show any signal, since PrP\(^c\) was fully digested.

**Positive samples** were also all treated with proteinase K.

**BSE positive bovine sample** (position 2), **classical scrapie positive sample** (position 6), and **CWD positive sample** (position 4) show a typical 3 band pattern, demonstrating digestion of PrP\(^c\) and transformation of the disease specific prion protein into a proteinase resistant core fragment with reduced molecular mass following removal of the N-terminus part of the protein. The two higher bands correspond to mono and di-glycosylated forms (27-30 kDa) while the lower band corresponds to the non glycosylated form.

**Ovine sample experimentally infected with BSE** (position 5) is presenting a higher signal on the di-glycosylated than on the mono-glycosylated band. Nevertheless, this typical glycoprofile can not be considered as a sufficient proof the infection of the animal with BSE. According to the Community Reference Laboratory (CRL) recommendations, a differentiation assay must be performed on this type of sample to conclude between scrapie and BSE. Please contact Bio-Rad for more information on the Bio-Rad Discriminatory test.
Atypical scrapie (e.g. Nor98) affected ovine sample (position 8) is presenting an atypical glycoprofile. A lower band is visible at approximately 12 kDa, while other upper bands are not located at the same positions compare to “typical” scrapie cases. Signal is also stronger on the lowest band than on the upper band.

Gel reading must be considered cautiously since a strong positive sample detected with the TeSeE™ Western Blot assay may hide the nearest negative or low positive sample.

Limits of the test:
A negative result means that the test sample does not contain detectable PrPres by TeSeE™ Western Blot assay. However, as very low levels of PrPres may not be detected, such a result does not exclude the possibility of infection.

Any sample with a negative result according to the TeSeE™ Western Blot assay interpretation criteria, following a positive rapid test result, should be tested with one of the other OIE certified confirmatory methods, Immunohistochemistry (IHC) or SAF-Immunoblot.

Any sample with a reproducible positive result according to the test interpretation criteria must be verified in accordance with current legal regulation.

8 - PRECAUTIONS

The quality of the data obtained depends on compliance with the following good laboratory practices:
• Reagents must be stored at the appropriate temperature (refer to supplier’s indications).
• Do not use reagents whose shelf-life has expired.
• Do not use reconstituted proteinase K after 10 hours storage at room temperature (+18°C to +30°C).
• Do not mix or combine reagents derived from different batches of the TeSeE™ Western Blot assay during the same manipulation, with the exception of grinding tubes, reagent A, reagent B and proteinase K.
• Allow the reagents and buffers to adjust to room temperature (+18°C to +30°C) for 30 minutes before use.
• Thoroughly reconstitute reagents, avoiding any contamination.
• Do not perform the test in the presence of reactive vapors (acids, alkalines, aldehydes) or dust, which could alter the enzymatic activity of the conjugate.
• The enzymatic reaction is very sensitive to all metals or metallic ions. Consequently, no metallic element must be in contact with the conjugate.
• Only use polypropylene tubes.
• Use clean glassware, rinsed in distilled water, or preferably disposable material.
• Use a new pipette tip for each sample.
• When starting electrophoresis and transfer, check that the 2 electrodes are in contact with buffer.
• All the rinsing times must be respected to avoid any excess background noise during final staining with ECL reagent.

9 - HYGIENE AND SAFETY INSTRUCTIONS

Generally, hygiene conditions, biosafety measures and good laboratory practices must be in agreement with the recommendations of national regulatory authorities.
• All reagents of the kit are intended for use in “in vitro” diagnosis.
• Wear disposable gloves when handling reagents and samples and wash your hands thoroughly after handling them.
• Do not pipette by mouth.
• Use polypropylene containers to avoid broken glass.
• All the materials directly in contact with the samples and the wash solutions must be considered as contaminated.
• Avoid splashing samples or solutions containing samples.
• Contaminated surfaces must be cleaned with 20 000 ppm sodium hypochlorite solution (bleach). When the contaminating liquid is an acid, contaminated surfaces must be first neutralized with sodium hydroxide before using bleach. Surfaces must be rinsed with distilled water, dried with ethanol and wiped with absorbent paper. The material used for cleaning must be discarded in a specific container for contaminated waste.
• Samples, material and contaminated products must be eliminated after decontamination:
  - either by soaking in 1 M sodium hydroxide (final concentration) for at least 1 hour at room temperature (+18°C to +30°C),
  - or by soaking in 20 000 ppm sodium hypochlorite solution for at least 1 hour at room temperature (+18°C to +30°C),
  - or by autoclaving at 134°C minimum for at least 18 minutes, under 3 bars of pressure.
  
  **Note:** never autoclave solutions containing bleach or reagent B.
• All operations involved in Transmissible Spongiform Encephalopathy (TSE) screening tests are subject to regulations and must be performed in an isolated, limited and controlled access laboratory devoted exclusively to this activity. A laboratory coat, overshoes, gloves, mask with visor or simple mask with safety glasses are required to ensure the operator’s safety.

• Operators must receive specific training concerning the risks related to TSEs agents or prions and the validated modes of decontamination for unconventional agents. Biosafety measures must be in agreement with recommendations of regular authorities of the country.

• Neutralize and/or autoclave all wash solutions or wash wastes or any liquid containing biological samples prior to their elimination.

• For hazard and precaution recommendations relating to this test kit, please refer to the pictogram(s) displayed on reagent labels and the information supplied at the end of this instructions for use document. The Safety Data Sheet is available on www.bio-rad.com.

10 - REFERENCES


   Assessment of Western immunoblotting for the confirmatory diagnosis of ovine scrapie and bovine spongiform encephalopathy (BSE) - Journal of Veterinary Diagnostic Investigations, 4, 447-449.


   Screening slaughtered cattle for BSE - Nature: 409; 476-477.
Cases of scrapie with unusual features in Norway and designation of new type, Nor98. Veterinary Record 153, 202-208.


Atypical scrapie cases in Germany and France are identified by discrepant reaction patterns in BSE rapid tests. Journal of Virological Methods 117, 27-36.


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Tässä tuotteessa on ihmisestä tai eläimistä peräisin olevia osia. Käsittele varovasti.

Ce produit contient des composants d’origine humaine ou animale. Manipuler avec précaution.

Αυτό το προϊόν περιέχει ανθρώπινα ή ζωικά στοιχεία. Χειριστείτε το με προσοχή.

Ovaj proizvod sadrži ljudske ili životinjske sastojke. Pažljivo rukovati.

A készítmény emberi vagy állati eredetű összetevőket tartalmaz. Óvatosan kezelendő.

Questo prodotto contiene componenti umane o animali. Maneggiare con cura.

本製品にはヒトまたは動物由来の構成成分が含まれます。取り扱いにご注意下さい。

본 제품은 사람 또는 동물유래의 성분이 포함되어 있습니다. 취급에 주의하시기 바랍니다.

Dan il-prodott fih komponenti umani jew tal-animali. Uża b’attenzjoni.

Dit product bevat menselijke of dierlijke bestanddelen. Breekbaar.

Dette produktet inneholder humane eller animalske komponenter. Hanteres med forsiktighet.

Niniejszy produkt zawiera składniki pochodzenia ludzkiego lub zwierzęcego. Należy obchodzić się z nim ostrożnie.

Este medicamento contém componentes de origem humana ou animal. Manuseie com cuidado.

Aceast produs conține materiale de origine umană sau animală. Manevrați-l cu grijă.

Denna produkt innehåller beståndsdelar från människa eller djur. Hantera produkten varsamt.

Izdelek vsebuje človeške ali živalske sestavine. Rokujte previdno.

Tento výrobok obsahuje l'udské alebo zvieracie zložky. Narábjte s ním opatrne.
опасно
Запалимо течност и пари. Предизвиква сериозно увреждане на очите. Предизвиква дразнене на кожата. Може да причини алергични или астматични симптоми или затруднения в дишането при вдишване. Вреден за водните организми, с долуходобими улъжки.
Да се пази от топлина. Тютюнопушенето забранено. Избягвайте вдишване на прах/пушек/газ/дим/изпарения/аерозоли. Използвайте предпазни ръкавици/предпазно облекло/предпазни очила/предпазна маска за лице.
ПРИ КОНТАКТ С ОЧИ: Непълено промивайте няколко минути. Свалете контактните лещи, ако има такива и доколкото това е възможно. Продължавайтеда промивате.
PРИ КОНТАКТ С КОЖА: Измийте обилно със сапун и вода.
Да се избяга изпускане в околната среда. Изхвърлете съдържанието/контейнера в съответствие с местните/регионални/национални/международните разпоредби.

(CZ)
Nebezpečí

(DE)
Gefahr

(DK)
Fare
Flammable liquid and vapour. Causes serious eye damage. May cause skin irritation. May cause respiratory difficulties if inhaled. Harmful to aquatic life with long lasting effects. Keep away from heat/sparks/open flames/hot surfaces. No smoking. Avoid breathing dust/fume/gas/mist/vapours/spray. Wear protective gloves/eye protection/face protection. IN CASE OF CONTACT WITH EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN: Wash with plenty of soap and water. Avoid release to the environment. Dispose of contents/container in accordance with local/Regional/national/international regulations.
Zapaljiva tekućina i para. Uzrokuje teške ozljede oka. Nadražuje kožu. Ako se udiše može izazvati simptome alergije ili astme ili teškoće u dišanju.

Čuvati odvojeno od topline/iskre/otvorenog plamen/vrućih površina. – Ne pušiti. Izbjegavati ispuštanje u okoliš.

&bull; Nositi zaštitne rukavice/zaštitnu odijelo/zaštitu za oči/zaštitu za lice.
&bull; U slučaju dodira s očima: oprezno ispirati vodom nekoliko minuta. Ukloniti távol tartandó. Tilos a dohányzás.

&bull; Teniti ispiranje.
&bull; Ha bőrre került, hosszú idejű károsodást okoz.

&bull; Nehimzó szemállomás és őz következmények, és összegyűlt üveg.

&bull; Nehimzó rúd és forró felületek.

&bull; Izbjegavati ispuštanje u okoliš.

&bull; Izbjegavati izlaganje u okoliš.

&bull; Nastaviti ispiranje.

&bull; U slučaju dodira kontaktne leće ukoliko ih nosite i ako se one lako por (pusťte).

&bull; Ne pušiti. Izbjegavati ispuštanje u okoliš.

&bull; Odložite sadržaje u skladu s lokalnim/regionalnim/nacionalnim međunarodnim odredbama.

Kerüls:

&bull; Lemosás bősszúval.

&bull; Konakos lieptelės ir gaisras.

&bull; Hőtől/szikrától/nyílt lángtól/forró felületektől hosszú idejű károsodást okoz.

&bull; Penfolyadék és gőz.

&bull; Veszély

&bull; Nekinek az anyagnak a környezetbe való kijutását.

&bull; Tulajdonosok, kompjúter vagy más elektronikus készülékek.

&bull; Išimti kontaktinį lęšį, jei jis yra ir jei jis yra lengvai išimtas.

&bull; Patekus į akis: keletas minutes atsargiai plauti akis. Išimti kontaktinio lešiojimo, jei jis įprastai nėra ir jei jis lengvai išimtas.

&bull; Patekus į akis: daugiau negu miškas ir nuostabiai išdamsiai.

&bull; Į akis: kelias minutes atsargiai plauti akis. Patekus į akis: daugiau negu miškas ir nuostabiai išdamsiai.

&bull; Į akis: kelias minutes atsargiai plauti akis. Patekus į akis: daugiau negu miškas ir nuostabiai išdamsiai.

&bull; Į akis: kelias minutes atsargiai plauti akis. Patekus į akis: daugiau negu miškas ir nuostabiai išdamsiai.

(NO)

Fare

(PL)

Niebezpieczeństwo

(PT)

Perigo
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