PrioWESTERN BSE Kit

Test for *in vitro* detection of TSE-related PrP^{Sc}

Catalog Number PR12000 **Pub. No.** MAN0013785 **Rev.** A.0

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.

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Producers of TSE rapid tests must have a quality assurance system in place, agreed by the European Union Reference Laboratory (EURL), which ensures that test performance does not change. Sampling tools and modifications to the rapid test or to the test protocol (including sampling) may only be made following advance notification to the EURL and will only be granted provided that the EURL finds that the modification does not reduce the sensitivity, specificity or reliability of the rapid test. Following EURL approval, details of the modification, shall be communicated to the Commission and to European Union National Reference Laboratories.

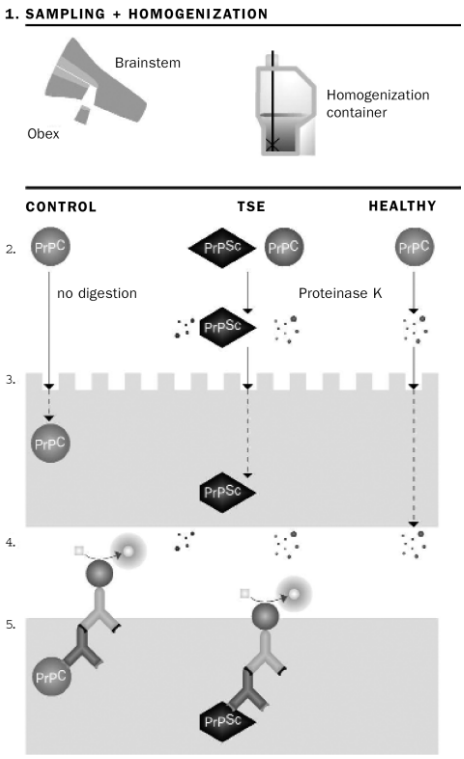
Introduction

Various tissues of a prion-infected animal contain a pathologically altered, disease specific form of the prion protein, PrP. The altered prion protein is denominated PrP^{Sc}. The normal isoform of PrP is termed PrP^{C} (the cellular form of PrP).
PrP^{Sc} differs from PrP^{C} in its protease resistance: Upon treatment with Proteinase K, PrP^{C} is degraded, while PrP^{Sc} is reduced from its original size of 32–35 kD to a smaller size of 27–30 kD. The remaining protease-resistant PrP^{Sc} fragment is referred to as PrP27-30.
The Applied Biosystems™ PrioWESTERN BSE Kit achieves its high precision and reliability by monitoring three independent criteria: protease-resistance, glycosylation pattern and lower molecular weight of the protease-resistant PrP^{Sc}-fragment (27–30 kD) compared to normal, undigested PrP.
The unique properties of the buffer solutions used in PrioWESTERN BSE Kit and the high affinity of the antibody allow that the test can be performed directly with tissue homogenates combining the reliability of the Western blotting procedure with the speed needed for mass screening.
The PrioWESTERN BSE Kit was the first BSE-test kit to be approved by the Swiss authorities in 1998. In 1999 it was officially acknowledged by the EU as the only test to achieve 100% sensitivity and 100% specificity without retesting.
In the CRL Analytical sensitivity study in 2009 was concluded that PrioWESTERN BSE Kit performed with a maximal 2 $\log_{10}$ inferiority range as compared to the most sensitive test system.
The validation data for this kit have been certified by the OIE, based on expert review, as fit for the following purposes:
Fit for the post-mortem diagnosis of bovine spongiform encephalopathy in cattle and for the following purposes:

1. To confirm diagnosis of suspect or clinical cases (includes confirmation of a positive screening test);
2. To estimate prevalence of infection to facilitate risk analysis (surveys/herd health schemes/disease control, e.g. surveys, implementation of disease control measures) and to assist in the demonstration of the efficiency of control policies;
3. To confirm a non-negative test result obtained during active surveillance with a different type of test.

Test principle

After Sample Collection and Registration, samples are analyzed with the PrioWESTERN BSE Kit. The PrioWESTERN BSE Kit follows a five step protocol, consisting of Homogenization, Protease Digestion, Gel Electrophoresis, Blotting and Immunological Detection. One person can process 100 samples (duplicate assays) within 6–8 hours.
Samples are collected, registered, and a homogenate is prepared from a defined piece of brain tissue. Treatment with Proteinase K degrades PrP^{C} completely while PrP^{Sc} is reduced to the 27–30 kD fragment. The proteolytic reaction is stopped, and PrP^{Sc} is detected in the PrioWESTERN BSE Kit assay. Digested homogenates are subjected to gel electrophoresis and Western blotting. The blot membranes are incubated with a monoclonal antibody—with high affinity for PrP—for the detection of protease resistant PrP^{Sc}. The signal is visualized using the secondary antibody-alkaline phosphatase (AP) conjugate.



Kit components *(Continued on next page)*

Kit for 100 samples (duplicate analyses). Shelf life of all un-opened components is 1 year after production if stored at 5±3°C. See kit label for actual expiry date. The shelf life of diluted, opened or reconstituted components is noted below, when appropriate.

Component	Description
1: Homogenisation Buffer Concentrate (5x)	5x concentrate, dilute before use. One bottle containing 200 mL of 5x concentrated Homogenization Buffer. Prepare 1x homogenization working solution by mixing 1 part Homogenisation Buffer (5x) with 4 parts purified water. Shelf life of the homogenization working solution: 1 week at 5±3°C.
2: Digestion Buffer (1x) (Cap color code: yellow)	Ready-to-use. One vial containing 4 mL of Digestion Buffer.
3: Proteinase K (Cap color code: white)	Ready-to-use. One vial containing 4 mL of Proteinase K.
4: Digestion-Stop (1x) (Cap color code: red)	Ready-to-use. One vial containing 4 mL of Proteinase K blocker to stop proteolytic activity of the Proteinase K.
5: Control Sample	Ready-to-use. One vial containing 200 µL functional control (normal PrP^{C}) and molecular weight markers (97/66/45/30/20/14 kD) in PAGE Sample Buffer. Mix before use, e.g. by flicking the tube.
6: PAGE Sample Buffer (1x)	Ready-to-use. One vial containing 25 mL of Sample Buffer for SDS Polyacrylamide Gel Electrophoresis (PAGE). (Contains 2-mercapto-ethanol. Opened vials release a bad smell. However, even if 100 vials are opened simultaneously in a normal aerated room, air concentrations do not reach the Workplace Environmental Exposure Level of 0.65 mg/m³ defined by the American Industrial Hygiene Association.)

Component	Description
7: PVDF Blocking Buffer Concentrate (5x)	5x concentrate, dilute before use. One bottle containing 100 mL of concentrated Blocking Buffer to block unspecific binding sites. Dilute 100 mL of Blocking Buffer with purified water to a final volume of 0.5 liter.
8: 1. Antibody 6H4	One vial containing 30 µL of monoclonal antibody to PrP (mouse anti-PrP IgG1). Working dilution: 1:5000. (In case fluid sticks to wall or lid, the tube can be centrifuged).
9: 2. Antibody-AP	One vial containing 30 µL of goat anti-mouse IgG-AP, an antibody to mouse IgG that is conjugated to alkaline phosphatase. Working dilution: 1:5000. (In case fluid sticks to wall or lid, the tube can be centrifuged).
10: Luminescence Buffer Concentrate (10x)	10x concentrate, dilute before use. One bottle containing 27 mL of Luminescence Buffer concentrate. Dilute with purified water to 270 mL before use.
Additional kit contents	• Package Insert • Labels for working solutions

Additional material required

Unless otherwise indicated, all materials are available through **thermofisher.com**. The **highlighted** items have been validated for the use with the PrioWESTERN BSE Kit. The use of different devices is in the responsibility of the user.

Use	Description
General	Laboratory equipment according to national safety regulations • Purified water: at least equivalent to Grade 3 water as defined by ISO 3696:1987 (E) • Single channel pipette (1–10 µL) • Single channel pipette (10–100 µL) • Single channel pipette (100–1000 µL) • Single channel pipette (1–5 mL) • Multichannel pipette (0.5–10 µL) • Multichannel pipette (10–100 µL) • Pipette tips (as recommended by pipette manufacturer) • Solution reservoirs • Incubation trays • 15 mL conical tubes • 50 mL conical tubes
Homoge-nization	• Cutting tool and forceps • Balance • Dispenser for homogenization working solution • 1.2 mL 96-deep well plate (used as sample Master Plate) • **PrioGENIZER™** homogenization device with six racks and one tray (Cat. No. PR10000) and **PrioCLIP™** homogenization containers (Cat. No. PR10010) or **FASTH/MediFASTH or FASTH 2** homogenization device and **Prypcon** homogenization containers (Syntec International) or **Omni Tissue Homogenizer** (TH115, TH220) and **Soft Tissue Omni Tip™ Plastic Homogenizing Probes** (32750) (Omni International)
Protease digestion	• 96-well microplates (0.2 mL wells; used as Digestion Plate) • Sealing film • Microplate incubator (reaching at least 100°C)
Gel electro-phoresis	• **NuPAGE™ 12% Bis-Tris Protein Gels** (17 slots) (Cat. No. NP0349BOX) • **NuPAGE™ MOPS SDS Running Buffer** (20x) (Cat. No. NP0001 or NP000102) • **NuPAGE™ Antioxidant** (Cat. No. NP0005)
Blotting	• **PVDF membrane, Immobilon-P, 0.45 µm** (EMD Millipore) • Methanol (approx. 98%) • Transfer Buffer (10x): 30.28 g Tris base/144.13 g Glycine/ add purified water to 1000 mL
Immuno-logical detection	• Tris-Buffered-Saline (TBS, pH 7.4): 8 g NaCl/ 0.2 g KCl/ 3 g Tris base Add purified water to 1000 mL, adjust pH to 7.4 with HCl • Tris-Buffered-Saline with Tween (TBST): TBS with 0.05% (v/v) Tween–20 • Ponceau S (20x): 0.5% (w/v) Ponceau S/ 5% (v/v) acetic acid. Dilute with TBST to 1x for use • CDP-Star concentrate (= Alkaline Phosphatase Substrate) (12.5 mM; Cat. No. T2310) or Roche Diagnostics GmbH (25 mM) or CDP-Star, Ready-To-Use (0.25 mM; Cat. No. T2146) • X-Ray films

Test procedure

Precautions

- National Safety Regulations must be strictly followed.
- The PrioWESTERN BSE Kit must be performed in laboratories suited for this purpose.
- Persons performing the test have to be trained generally in working with prions and specifically in performing the PrioWESTERN BSE Kit.
- Samples should be considered as potentially infectious and all items which were in contact with the samples as potentially contaminated.

Notes

To achieve optimal results with the PrioWESTERN BSE Kit, the following aspects must be considered:

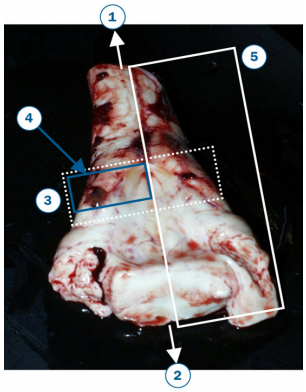
- **The Test Procedure protocol must be strictly followed.**
- Pipette tips have to be changed for every pipetting step.
- The use of either pipette filter tips or separate pipettes for the different pipetting steps is strongly recommended. In addition, the accuracy of pipettes should be calibrated regularly. National guidelines apply.
- Separate solution reservoirs must be used for each reagent.
- Kit components must not be used after their expiry date or if changes in their appearance are observed.
- Kit components of different kit lot numbers must not be used together.
- Non-disposable cutting tools and forceps must be decontaminated according to guidelines enforced by national authorities.
- When the PrioGENIZER™ is used for homogenization, only program P0 PRIONICS TSE must be used for homogenization of brain tissue.

Sampling and homogenization

- Take 0.45–0.70 g nervous tissue from the preferred area of the left **or** the right side of the brainstem with e.g. a scalpel.

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 Standards for Diagnostic Test 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 picture below shows the sampling area within box 4.

Note: After sample collection, a complete hemi-section of the brain stem with an intact obex region must remain available for confirmatory testing. (For a detailed sampling protocol contact Technical Support.)



(1) Spinal cord
(2) Brain
(3) Obex region
(4) Area to be used for PrioWESTERN BSE Kit testing
(5) Area to be used by the BSE Reference Center

Medulla oblongata
The tissue sample is an approx. 8 cm long piece of brainstem/cervical spinal cord.

Homogenization

Preparatory steps

- Dilute 5x Homogenization Buffer (Component 1) with purified water to prepare homogenization working solution (Appendix A).

Homogenization

- Transfer sample to a homogenization container and determine weight on balance (0.45–0.70 g).
- Add ten volumes of homogenization working solution (w/v; e.g. 5 mL to 0.50 g brain tissue) and homogenize sample using the PrioGENIZER™ (Program P0 Prionics TSE), the FASTH/ MediFASTH/ FASTH 2 (45±5 seconds, 20,000±1,000 rpm) or the Omni Tissue Homogenizer (60±10 seconds at maximum speed) homogenization device.
- Store **two** 1 mL samples per homogenate in a 96-well sample Master Plate. (From now on, each step will be done with two samples per original homogenate).
- PrioCLIP™ and Prypcon homogenization containers of samples tested "TSE negative" may be washed for reuse (see PrioCLIP™/Prypcon Wash Protocol, Appendix D).

Protease digestion

Following amounts are for 48 samples.
(See Appendix B for volumes needed for samples numbers other than 48.)

Preparatory steps

- Set the temperature of the microplate incubator to 48±1°C approx. 1 hour prior to use.
- Add 10 µL of Digestion buffer (Component 2) to each well of the Digestion Plate.

Protease digestion

- Transfer 100 µL (mix first by pipetting up and down at least three times) of each homogenate from the Master Plate to the corresponding well of the Digestion Plate with a multichannel pipette. Afterwards, the Master Plate may be covered and stored at –20°C to –80°C for up to 12 months.
- Add 10 µL of Proteinase K (Component 3) to each well of the Digestion Plate and mix by pipetting up and down at least three times.
- Cover the Digestion Plate with a Sealing Film.
- Digest for 40±1 minutes at 48±1°C.
- Stop the reaction by adding 10 µL Digestion Stop (Component 4). Mix by pipetting up and down at least three times.

Gel electrophoresis

Preparatory steps

- Mount 17-slot 12% NuPAGE™ gels: Carefully remove the comb and white plastic foil at the bottom of the gel.
- Heat Control Sample (Component 5) to 65±3°C for 2–5 minutes.
- Set the temperature of the microplate incubator to 98±4°C approx. 1 hour prior to use.

Gel electrophoresis

- Add 100 µL of PAGE Sample Buffer (Component 6) to the digested homogenate in the Digestion Plate and mix by pipetting up and down at least three times.
- Boil samples at 98±4°C for 5 minutes±30 seconds.
- The Digestion Plate may be covered with a Sealing Film and stored at –20°C to –80°C for up to 5 days.
- Previously prepared samples are heated to 65±3°C for 2–5 minutes before loading.

Sample loading

- Load 10 µL of the Control Sample in the first lane.
- Load 10 µL of the heated samples per lane.
- Fill up inner and outer chamber with 1x NuPAGE™ SDS-MOPS Running Buffer and add 500 µL NuPAGE™ Antioxidant to the **inner** chamber only.

Electrophoresis

- Run loaded gels at 200 V until the dye front is about 1–2 cm from the bottom of the gel (approx. 30 minutes).

Blotting

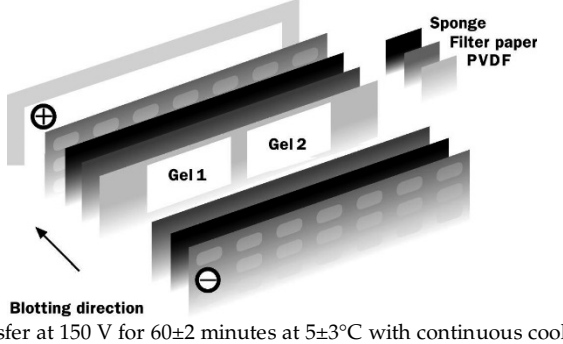
Preparatory steps

- Wet the PVDF membrane (EMD Millipore, Immobilon-P, 0.45 µm, 13 × 17 cm) in methanol (approx. 98%) for a few seconds. Equilibrate the membrane for at least 10 minutes in 1x Transfer Buffer (see Appendix B for volumes needed).
- Fill transfer unit with pre-chilled (5±3°C) 1x Transfer Buffer.

Blotting

Sandwich assembly

- Place membrane on Whatman Paper moistened with 1x Transfer Buffer or purified water.
- Open plastic frame of NuPAGE™ gel. Remove the top part of the gel containing the slots and the bottom part below the dye front. Place the gel on the membrane (avoid air-bubbles). Up to 6 gels can be placed on one membrane of the above size (see Appendix C).
- Overlay gels with moistened Whatman Paper, place sponge on top.
- Close transfer cassette and place in transfer unit. Proteins are negatively charged and move towards the positive (red) pole of the transfer unit. Make sure that the cassette is inserted with the PVDF membrane towards the positive pole and the gels towards the negative pole.



- Transfer at 150 V for 60±2 minutes at 5±3°C with continuous cooling.
- Remove the membrane and stain bound proteins with 1x Ponceau S. Label the position of the size markers. Destaining is performed with TBST until the red color has disappeared (approx. 2 × 1 minutes).

Immunological detection

Blocking

- Incubate the membrane in a plastic incubation tray with 50±2 mL of 1x PVDF Blocking Buffer (Component 7; see Appendix A for the dilution table) for 35±5 minutes at 22±3°C on a rocking platform with gentle agitation.

1st Antibody

- Dilute 10 µL of 1. Antibody 6H4 (Component 8) in 50±2 mL of TBST (1:5000 dilution), add to membrane and incubate for 60±5 minutes at 22±3°C (or alternatively for 12–18 hours at 5±3°C) with gentle agitation on a rocking platform.
- Wash membranes 3x for approx. 5 minutes with TBST.

2nd Antibody

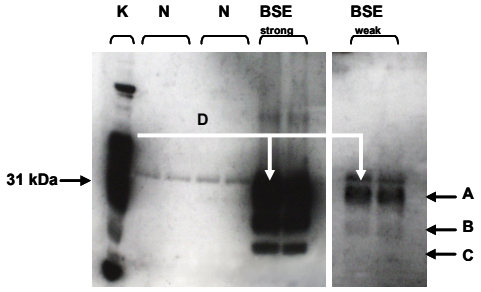
- Dilute 10 µL of 2. Antibody-AP (Component 9) in 50±2 mL of TBST (1:5000 dilution). Incubate for 30±1 minutes at 22±3°C with gentle agitation.
- Wash membranes 5x for approx. 5 minutes with TBST.

Detection

- Equilibrate membrane for 5–10 minutes in 50±2 mL 1x Luminescence Buffer (Component 10; see Appendix A for the dilution table).
- Dilute 100 µL CDP-Star (12.5 mM; 50x) or 50 µL (25 mM; 100x) in 5 mL 1x Luminescence Buffer.
- Place the membrane on a glass plate. Distribute 5 mL of the diluted CDP-Star solution or 5 mL of the ready-to-use solution evenly over the membrane and incubate for 5±1 minutes at 22±3°C.
- Remove excess liquid. Remove remaining liquid from the membrane with a soft Kleenex tissue and immediately cover the membrane with Saran foil. Expose the membrane to an X-Ray film until a strong signal of the positive control and either the background or the Proteinase K bands are visible (approx. 5 up to 20 minutes). Expose longer or shorter times for optimal signal visualization. Alternatively use a CCD-Camera Detection System (e.g. FluorChem™, Alpha Innotech Corp.).

Interpretation of results

The following figure shows the expected band patterns of BSE-negative, BSE-positive and control samples, respectively. The control sample (K) contains the normal isoform of the prion protein (PrP^C) which is visualized via immunological detection. The corresponding diffuse band is spread from 25–35 kD due to glycosylation of PrP^C which causes a heterogeneous distribution.



Negative samples (N) do not show a specific signal. The 31 kD band (not always visible) results from unspecific binding of the secondary antibody to Proteinase K and can be used as an orientation aid.

Positive samples (BSE_{strong}; BSE_{weak}) exhibit a signal consisting of three bands, the top one (A) corresponding to a protein with an approximate molecular weight of 30 kD. The signal intensity of all bands (in particular that of the lower bands B and C) can be weaker than depicted here, but the top band (A) should be clearly visible. The arrow (D) illustrates the difference in molecular weight between digested, pathological prion protein and the undigested, normal protein.

If this test is to be used for screening, a repeat reactive sample must be confirmed in a National Reference Laboratory using an additional confirmatory method. If used for confirmation this test can only be used in conjunction with OIE/CRL recommendations.

Appendix A – Tables for preparation of working solutions

Homogenization working solution

Mix indicated volumes of purified pure water and 5x Homogenization Buffer (Component 1) to obtain the desired volume of homogenization working solution:

Shelf life of homogenization working solution: 1 week at 5±3°C.

Vol. of homogenization working solution		Volume of Homogenization Buffer (5x) (Component 1)		Volume of purified water
250 mL	=	50 mL	+	200 mL
500 mL	=	100 mL	+	400 mL
1000 mL	=	200 mL	+	800 mL

PVDF Blocking Buffer

Vol. of PVDF Blocking Buffer (1x)		Vol. of PVDF Blocking Buffer (5x) (Component 7)		Vol. of purified water
500 mL	=	100 mL	+	400 mL

Luminescence Buffer

Mix indicated volumes of purified water and 10x Luminescence Buffer (Component 6) to obtain the desired volume of Luminescence Buffer (1x):

Vol. of Luminescence Buffer (1x)		Vol. of Luminescence Buffer (10x) (Component 10)		Vol. of purified water
270 mL	=	27 mL	+	243 mL

NuPAGE™ SDS-MOPS Running Buffer 1x

Vol. of NuPAGE™ SDS-MOPS Running Buffer (1x)		Vol. of NuPAGE™ SDS-MOPS Running Buffer (20x)		Vol. of purified water
1000 mL	=	50 mL	+	950 mL

Transfer Buffer

Vol. of Transfer Buffer (1x)		Vol. of Transfer Buffer (10x)		Vol. of purified water		Vol. of methanol (98%)
2000 mL	=	200 mL	+	1600 mL	+	200 mL

TBST

Vol. of TBST (1x)		Vol. of TBS (20x)		Vol. of purified water		50% Tween 20
1000 mL	=	50 mL	+	950 mL	+	1 mL

Ponceau S

Vol. of Ponceau S (1x)		Vol. of Ponceau S (20x)		Vol. TBST (1x)
1000 mL	=	50 mL	+	950 mL

Appendix B – Volumes needed for different numbers of samples

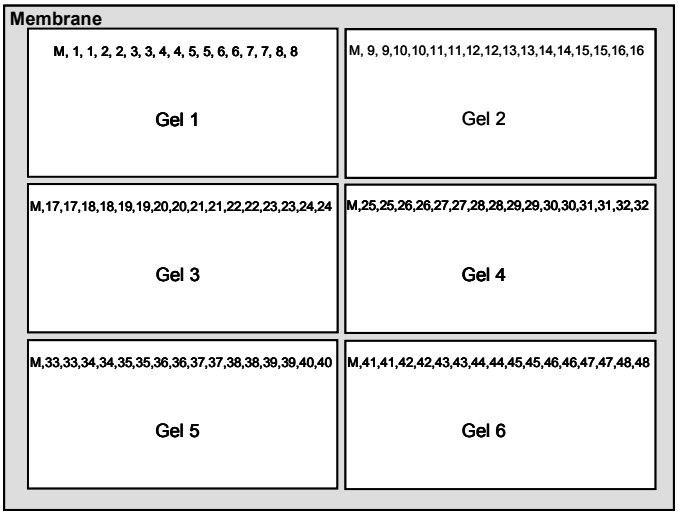
No. of gels	Tray size (cm)	Membrane size	TBST	1. Antibody
6	Large (22.4 × 15.6)	13 × 17 cm	50 mL	10 µL
4	Large (22.4 × 15.6)	9 × 17 cm	50 mL	10 µL
3	Medium (15 × 11.4)	13 × 8.5 cm	25 mL	5 µL
2	Medium (15 × 11.4)	9 × 8.5 cm	25 mL	5 µL
1	Small (5.5 × 9.5)	4.5 × 8.5 cm	10 mL	2 µL

Tray size (cm)	TBST	2. Antibody	Luminescence Buffer	CDP-Star	
				12.5 mM	25 mM
Large (22.4 × 15.6)	50 mL	10 µL	5 mL	100 µL	50 µL
Large (22.4 × 15.6)	50 mL	10 µL	4 mL	80 µL	40 µL
Medium (15 × 11.4)	25 mL	5 µL	3 mL	60 µL	30 µL
Medium (15 × 11.4)	25 mL	5 µL	3 mL	60 µL	30 µL
Small (5.5 × 9.5)	10 mL	2 µL	2 mL	40 µL	20 µL

Appendix C – Scheme for placement of gels on blot membrane

Recommended scheme for placement of gels on blotting membrane:

- 96-well plate filled with 48 duplicate samples, transferred to six 17-slot gels. Numbers indicate samples 1–48.
- (M = molecular size marker with undigested PrP^{C})



Appendix D – PrioCLIP™/Prypcon Wash Protocol

General instructions

Sample traceability

PrioCLIP™/Prypcon homogenization containers must be labeled with sample number—using e.g. a waterproof pen or labels—to guarantee the sample traceability. Labeling of the containers can only be removed after release of results.

PrioCLIP™/Prypcon usage traceability

Homogenization containers should not be used more than 5 times. PrioCLIP™/Prypcon have to be labeled with dashes or dots using a waterproof pen after each use.

Do not use hypochlorite-containing disinfectants for washing.

Preparatory steps

- Fill two vessels with sufficient amounts of deionized water (at least 25 L) in order to allow complete submersion of the PrioCLIP™/Prypcon during the washing steps.

Draining

- Empty containers with homogenates tested "TSE negative" into an autoclavable, heat-resistant bottle or a disposable canister/flask.
- **Containers whose contents have been identified "initial reactive" must not be re-used and have to be disposed of according to the national safety guidelines.**

Washing

- Immerse the empty PrioCLIP™/Prypcon in a vessel with deionized water, rinse thoroughly.
- Inspect the homogenization containers visually for possible damage and tissue contamination during transfer from vessel one to vessel two. Discard any damaged or contaminated PrioCLIP™/Prypcon homogenization containers.
- Submerge containers and incubate at least 30 minutes at 22±3°C.

Drying

- Take the PrioCLIP™/Prypcon out of the vessel, shake out remaining water and let them dry completely at 22±3°C.
- Alternatively, PrioCLIP™/Prypcon can be dried in a heating/drying oven. Place the containers on a heat-resistant surface, heat them for 2 hours at 85±5°C and dry over night at approx. 50°C in a drying oven. Repeat heating step (2 hours, 85±5°C).
- Visually check PrioCLIP™/Prypcon. Discard containers that are damaged or contain remaining fluid or tissue.
- Now PrioCLIP™/Prypcon are ready for re-use.

Waste disposal

- Homogenates and washing solutions have to be disposed of according to national safety guidelines.

A detailed PrioCLIP™/Prypcon wash protocol (including pictures) can be requested from Technical Support.

Appendix E – OIE certification: Summary of validation studies

Analytical characteristics

Analytical sensitivity

- Dilution series were tested in the course of the European Union validation study. Of 20 positive homogenates tested at a 10^{-1} dilution, 15 scored positive, two doubtful and three negative. At the $10^{-1.5}$ dilution three samples were scored doubtful and the remainder negative. Two samples were also scored doubtful at 10^{-2} and a single sample at $10^{-2.5}$ dilution. [Positive samples were supplied by the Central Veterinary Laboratory (CVL), Weybridge: brainstem and spinal cord samples were selected from bovines showing clinical signs of BSE with confirmation by histological examination.]

Analytical specificity

- This has not been specifically examined. However some field studies using fallen stock samples (animals suffering from disorders other than BSE, e.g. encephalitis, brain oedema, rabies, listeriosis) have consistently shown that PrioWESTERN BSE Kit has a good analytical specificity [see D. Heim et al. (2000) Surveillance of BSE. *Arch Virol* Suppl. (16):127–33)].

Repeatability data

- Tests carried out over a period 2002 to 2007 show that the kit could detect a BSE positive sample at a 1:10 dilution.
- The repeatability was also studied in comparative testing of three BSE tests (Enfer – Bio-Rad TeSeE – PrioWESTERN BSE Kit) conducted by the EU Community Reference Laboratory for TSE (VLA, United Kingdom). The consistency between duplicate samples (n = 277) was high, except on two occasions when carryover from an adjacent positive well resulted in a small non-specific smudge in one of the adjacent wells of a negative sample. This was clearly different from positive results, which were of characteristic size, banding pattern and present in both duplicate wells.

Diagnostic characteristics

Threshold determination

This test does not give a quantitative reading. The response is qualitative and is based on the two criteria of the presence of the signal and its position. This allows easy discrimination between true positives and (potentially) false positives due to undigested normal PrP.

Diagnostic sensitivity (DSn) and specificity (DSp) estimates

Three external evaluation studies have been described for the estimation of diagnostic sensitivity and specificity:

- Tests performed at the Swiss BSE reference laboratory (Neurocenter/University of Bern) in February 2003 on 38 positive samples (18 homogenates & 20 tissue samples) from the UK / 190 negative samples from the Swiss BSE surveillance program (tissues samples from cattle older than 30 months that had tested negative by histology/immunohistochemistry).

	BSE positive by IHC	BSE negative by IHC
Test Positive by PrioWESTERN BSE Kit	**38**	**0**
Test Negative by PrioWESTERN BSE Kit	**0**	**190**
Diagnostic Sensitivity: 100%, CI [90.7–100.0%] **Diagnostic Specificity: 100%, CI [98.1–100.0%]**		

- Canadian field study, 2003. As a follow up to the detection of the index case in May, 2003, 2036 birth and feed cohorts were tested by immunohistochemistry (IHC) and the PrioWESTERN BSE Kit. This work was carried out by Canadian Food Inspection Agency laboratories at Lethbridge, Winnipeg, Nepean and St-Hyacinthe.

	BSE positive by IHC	BSE negative by IHC
Test Positive by PrioWESTERN BSE Kit	**1**	**0**
Test Negative by PrioWESTERN BSE Kit	**0**	**2036**
Diagnostic Specificity: 100%, CI [99.8–100.0%]		

- Evaluation of tests for the Diagnosis of Transmissible Spongiform Encephalopathy in Bovines for the European Commission, 1999. All samples were prepared by the EU Institute for Reference Materials and Measurements (IRMM) at Geed, Belgium and presented for testing in a coded 'blind' format to the different participants (PrioWESTERN BSE Kit was one of the four selected kits): A total of 300 positive samples (collected from cattle showing clinical signs of BSE and confirmed by histological examination - supplied by CVL Weybridge) and 1000 negative samples (collected in New Zealand from healthy adult cattle of at least 4 years of age and confirmed negative by histological examination).

	BSE positive by histology	BSE negative by histology
Test Positive by PrioWESTERN BSE Kit	**300**	**0**
Test Negative by PrioWESTERN BSE Kit	**0**	**1000**
Diagnostic Sensitivity: 100%, CI [98.8–100%] **Diagnostic Specificity: 100%, CI [99.6–100.0%]**		

Agreement between tests

The agreement of the PrioWESTERN BSE Kit with histological examination and IHC is high.

Reproducibility

During the EU field evaluation of 2004, samples were tested using the PrioWESTERN BSE Kit. These results were compared to the results achieved with other tests under evaluation. The samples were divided into three categories: positive samples, negative samples and samples of poor quality.

- A total of 335 BSE positive samples were tested in three labs (VLA, Newcastle, UK - Analytico Food BV, Heerenveen, Holland and Laboratorio Central de Veterinaria, Algete, Spain).
- A total of 24.534 BSE negative samples were tested in eight labs (Analytico Food BV, Heerenveen, Holland,; Institut für Veterinärmedizin, Mödling, Austria; Instituut voor Dierhouderij en Diergezondheid, Lelystad, Holland; Laboratorio EET, Leon, Spain; Labor Dr. Guenteert, Luzern, Switzerland; Instituto Zooprofilatiico Sperimentale del Piemonte, Turin, Italy; Irish Equine Centre, Kildare, Ireland; Arthus Biotech GmbH, Hamburg, Germany).
- An additional 423 samples of poor quality were tested in two labs (VLA, Newcastle, UK and NeuroCenter, Bern, Switzerland).

The inter-laboratory results of all samples tested were identical for the PrioWESTERN BSE Kit. Agreement with other tests under evaluation were very high with the exception of one weak positive sample detected in the PrioWESTERN BSE Kit that was not identified as positive when using the CediTect.

Source: Report: The Field Trial of Seven New Rapid Post Mortem Test for the Diagnosis of Bovine Spongiform Encephalopathy in Bovines; IRMM; 12 November 2004.

Applications

PrioWESTERN BSE Kit is currently in use in both reference and routine laboratories throughout the world.

Testing laboratories should participate in proficiency testing and laboratory training programmes organized by OIE Reference Laboratories.

Appendix F – Safety regulations

Laboratories **must** adhere to National Safety Regulations, but the following information – published by the Advisory Committee on Dangerous Pathogens (ACDP) – is available for guidance: "Transmissible spongiform encephalopathy agents: safe working and the prevention of infection", Department of Health, London, UK (can be ordered at the Stationery Office, ISBN 0113221665. An update is available under **https://www.gov.uk/government/publications/guidance-from-the-acdp-tse-risk-management-subgroup-formerly-tse-working-group**.

Appendix G - References

1. Oesch B, Doherr M, Heim D, Fischer K, Egli S, Bolliger S, Biffiger K, Schaller O, Vandevelde M, Moser M (2000) Application of Prionics Western blotting procedure to screen for BSE in cattle regularly slaughtered at Swiss abattoirs. *Arch Virol* 16:189–195.
2. Doherr MG, Oesch B, Moser M, Vandevelde M, Heim D (1999 Dec 4) Targeted surveillance for bovine spongiform encephalopathy. *Veterinary Record* 145:672.
3. Schaller O, Fatzer R, Stack M, Clark J, Cooley W, Biffiger K, Egli S, Doherr M, Vandevelde M, Heim D, Oesch B, Moser M (1999 Nov) Validation of a Western immunoblotting procedure for bovine PrPSc detection and its use as a rapid surveillance method for the diagnosis of bovine spongiform encephalopathy (BSE). *Acta Neuropathol (Berl)* 98(5):437–43.
4. Calavas D, Ducrot C, Baron T, Morignat E, Vinard JL, Biacabe AG, Madec JY, Bencsik A, Debeer S, Eliazsewicz M (2001 Jul 14) Prevalence of BSE in western France by screening cattle at risk: preliminary results of a pilot study. *Vet Rec* 149:55–56.
5. Heim D, Wilesmith JW (2000) Surveillance of BSE. *Arch Virol* (16):127–33.
6. (2012) Bovine Spongiform Encephalopathy. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* OIE Chapter 2.4.6.
7. European Food Safety Authority (EFSA), Parma, Italy (2009) Scientific Opinion on Analytical sensitivity of approved TSE rapid test, EFSA Panel on Biological Hazards (BIOHAZ). *EFSA Journal* 7(12):1436.

WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

Validated and certified by the OIE as fit for the purposes defined in this Package Insert.
Registration number: 20080102

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**
Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)
  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com /us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

Prionics Lelystad B.V. | Platinastraat 33 | 8211 AR Lelystad | The Netherlands

Revision history of Pub. No. MAN0013785 (English)

Rev.	Date	Description
A.0	3 April 2019	New document. Converted the legacy document (WESTERN_PI_v12.0_e_220213.doc) to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos.