Prionics®-Check WESTERN

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

Kit for 100 samples (duplicate analyses)

Version 10.0_e

Package Insert

For *in-vitro* veterinary diagnostic use only
Store at 5±3°C
Product No.: 12000

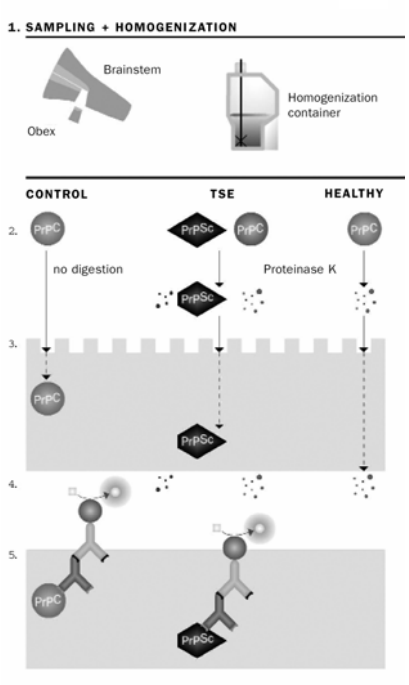
The producer of the rapid tests must have a quality assurance system in place agreed by the Community Reference Laboratory, which ensures that the test performance does not change. The producer must provide the test protocol to the Community Reference Laboratory. Sampling tools and modifications to the 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.

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 Prionics®-Check WESTERN 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 Prionics®-Check WESTERN 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 Prionics®-Check WESTERN 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.

Test Principle



After Sample Collection and Registration, samples are analyzed with the Prionics®-Check WESTERN. The Prionics®-Check WESTERN 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 Prionics®-Check WESTERN 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

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. Chemical hazard data are available in section "Safety Regulations and R&S Statements" (Appendix V).

Component 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.

Component 2
Digestion Buffer (1x) (Ready-to-use)
One vial containing 4 ml of Digestion Buffer.
Cap color code: yellow

Component 3
Proteinase K (Ready-to-use)
One vial containing 4 ml of Proteinase K.
Cap color code: white

Component 4
Digestion-Stop (1x) (Ready-to-use)
One vial containing 4 ml of Proteinase K blocker to stop proteolytic activity of the Proteinase K.
Cap color code: red

Component 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.

Component 6
PAGE Sample Buffer (1x) (Ready-to-use)
One vial containing 25 ml of Sample Buffer for SDS Polyacrylamide Gel Electrophoresis (PAGE).
(Contains 2-mercaptoethanol. 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/m^3 defined by the American Industrial Hygiene Association.)

Component 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.

Component 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).

Component 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).

Component 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

Prionics®-Check WESTERN

Additional Material Required

The **highlighted** items have been validated for the use with the Prionics®-Check WESTERN. The use of different devices is in the responsibility of the user.

Please also see our list "Prionics®-Check WESTERN additional material and devices" for more information (contact your local distributor or info@prionics.com).

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

Homogenization:
- Cutting tool and forceps
- Balance
- Dispensor 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 (Prionics AG, Product No: 10000) and **PrioCLIP™** homogenization containers (Prionics AG, Product No: 10010)

or

FASTH/MediFASTH or FASTH 2 homogenization device (Consul AR S.A:, Product No: 80040, 82040, 80020) and **Prypcon** homogenization containers (Consul AR S.A:, Product No: 80300)

or

Omnisystem homogenization device (Omni International Inc., Product No: TH220P) and **Omni tips** (Omni International Inc., Product No: 32750)

Protease Digestion:
- 96-well microplates (0.2 ml wells; used as Digestion Plate)
- Sealing film
- Microplate incubator (reaching at least 100 °C)

Gel Electrophoresis:
- **12% NuPAGE Gels (17 slots**) (Invitrogen™, Product No: NP0349BOX)
- **NuPAGE MOPS/SDS Running Buffer** (Invitrogen™, 500 ml: Product No: NP0001; 5 l: Product No: NP0001-02)
- **NuPAGE Antioxidant** (Invitrogen™, Product No. NP0005)

Blotting:
- **PVDF membrane, Immobilon-P 0.45 µm** (Millipore, Product No: IPVH 00010)
- Methanol (approx. 98%)
- Transfer Buffer (10x): 30.28 g Tris base/144.13 g Glycine/ add purified water to 1000 ml.

Immunological 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) (Applied Biosystems, 12.5 mM; Cat No. MSC050) or Roche Diagnostics GmbH, (25 mM; Cat No. 1759051) or CDP-Star, ready-to-use (Roche Diagnostics; Cat No. 2041677)
- X-Ray films

Test Procedure

Precautions
National guidelines for working with prions must be strictly followed (see also section "Safety Regulations and R&S Statements" Appendix V). The Prionics®-Check WESTERN 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 Prionics®-Check WESTERN.
Samples should be considered as potentially infectious and all items which were in contact with the samples as potentially contaminated.

Chemical hazard data are available in section "Safety Regulations and R&S Statements" (Appendix V).

Notes

To achieve optimal results with the Prionics®-Check WESTERN, the following aspects must be considered:

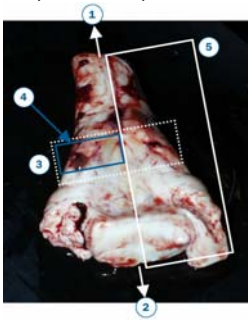
- **The Test Procedure protocol must be strictly followed.**
- Pipette tips have to be changed for every pipetting step.
- The use of either pipette filter tips or separate pipettes for the different pipetting steps is strongly recommended. In addition, the accuracy of pipettes should be calibrated regularly. 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.

Medulla oblongata
The tissue sample is an approx. 8 cm long piece of brainstem/cervical spinal cord.
(For a detailed sampling protocol contact info@prionics.com)



1) spinal cord
2) brain
3) obex region
4) area to be used for Prionics®-Check WESTERN testing
5) area to be used by the BSE Reference Center

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

HOMOGENIZATION:

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

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 sec ± 5 sec, 20'000 ± 1'000 rpm) or the Omnisystem (60 ± 10 sec 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 IV).

PROTEASE DIGESTION

Following amounts are for 48 samples
(See Appendix II 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 min 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 min.
- 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 min ± 30 s.
- 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 min 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 min).

BLOTTING

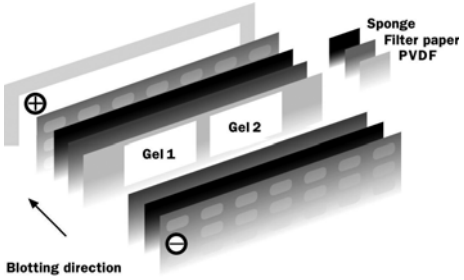
Preparatory Steps

- Wet the PVDF membrane (Millipore, Immobilon-P, 0.45 µm, 13 x 17 cm) in methanol (approx. 98%) for a few seconds. Equilibrate the membrane for at least 10 min in 1x Transfer Buffer (see Appendix II 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 III).
- 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 min 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 x 1 min).

IMMUNOLOGICAL DETECTION

Blocking:

- Incubate the membrane in a plastic incubation tray with 50±2 ml of 1x PVDF Blocking Buffer (Component 7; see Appendix I for the dilution table) for 35±5 min 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 min at 22±3°C (or alternatively for 12 -18 h at 5±3°C) with gentle agitation on a rocking platform.
- Wash membranes 3x for approx. 5 min with TBST.

2nd Antibody:

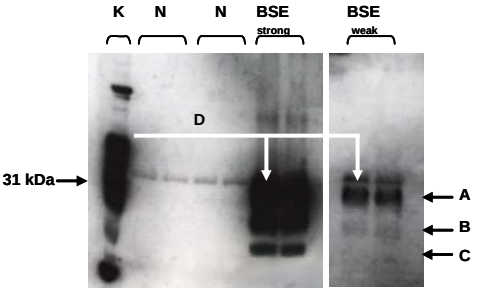
- Dilute 10 µl of 2. Antibody-AP (Component 9) in 50±2 ml of TBST (1:5000 dilution). Incubate for 30±1 min at 22±3°C with gentle agitation.
- Wash membranes 5x for approx. 5 min with TBST.

Detection:

- Equilibrate membrane for 5 - 10 min in 50±2 ml 1x Luminescence Buffer (Component 10; see Appendix I 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 min 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 min). 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.

General Remarks

Notice

This manual is believed to be complete and accurate at the time of publication. In no event shall Prionics AG be liable for incidental or consequential damage in connection with or arising from the use of this manual.

Liability

Prionics AG warrants its products will meet their applicable published specification when used in accordance with their applicable instructions and within the declared products life time. Prionics AG makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose. The warranty provided herein and the data, specifications and descriptions of Prionics AG products appearing in Prionics AG published catalogues and product literature may not be altered except by express written agreement signed by an officer of Prionics AG. Representation, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Prionics AG's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Prionics AG promptly of any such breach. If after exercising reasonable efforts, Prionics AG is unable to repair or replace the product or part, then Prionics AG shall refund to the customer all monies paid for such applicable product or part.

Prionics AG shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by any customer from the use of its products.

Prionics AG is an ISO 9001:2000 certified company.

Appendix I

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 Luminescence 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 (10x)		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 II

Volumes needed for different numbers of samples

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

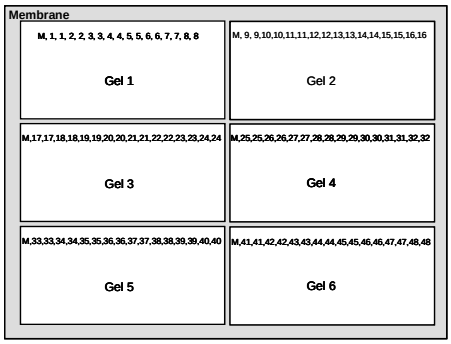
Tray size (cm)	TBST	2. Antibody	Luminescence Buffer	CDP-Star	
				12.5 mM	25 mM
Large (22.4 x 15.6)	50 ml	10 µl	5 ml	100 µl	50 µl
Large (22.4 x 15.6)	50 ml	10 µl	4 ml	80 µl	40 µl
Medium (15 x 11.4)	25 ml	5 µl	3 ml	60 µl	30 µl
Medium (15 x 11.4)	25 ml	5 µl	3 ml	60 µl	30 µl
Small (5.5 x 9.5)	10 ml	2 µl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

Appendix III

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 IV

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 de-ionized 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 de-ionized 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 min 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 hrs at 85±5°C and dry over night at approx. 50°C in a drying oven. Repeat heating step (2 hrs, 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 at info@prionics.com.

Appendix V

Safety Regulations and R&S Statements
Safety Regulations
1. National Safety Regulations must be strictly followed.
2. ACDP guidelines
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, phone number +44 (20) 7873 9090). An update is available under www.advisorybodies.doh.gov.uk/acdp/tseguidance/index.htm

R&S Statements

Component 1
Homogenization Buffer (5x)
Hazard Code: This product is not classified according to EU regulations.

Component 2
Digestion Buffer (1x)
Xn Harmful
Hazard Code:
R22 Harmful if swallowed.
R36/38 Irritating to eyes and skin.
S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S35 This material and its container must be disposed of in a safe way.
S36/37 Wear suitable protective clothing and gloves.

Component 3
Proteinase K
Hazard Code: This product is not classified according to EU regulations.

Component 4
Digestion Stop (1x)
Hazard Code: This product is not classified according to EU regulations.

Component 5
Control Sample
Hazard Code: This product is not classified according to EU regulations.

Component 6
PAGE Sample Buffer
C Corrosive
Hazard Code:
R21 Harmful in contact with skin.
R34 Causes burns.
R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S35 This material and its container must be disposed of in a safe way.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Component 7
PVDF Blocking Buffer Concentrate (5x)
Hazard Code: This product is not classified according to EU regulations.

Component 8
1. Antibody 6H4
Hazard Code: This product is not classified according to EU regulations.

Component 9
2. Antibody-AP
Hazard Code: This product is not classified according to EU regulations.

Component 10
Luminescence Buffer Concentrate (10x)
Hazard Code: This product is not classified according to EU regulations.

Contact

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