Prionics®-Check LIA
Bovine Spongiform Encephalopathy Antigen Test Kit, ELISA

Microplate-based Luminescence Immuno Assay for detection
of disease-specific prion protein in cattle (BSE)

Test kit for 400 samples
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Version 4.20e

Package Insert

For in-vitro veterinary diagnostic use only
Store at 5 ± 3 °C
Prod. No: (Prionics AG) 20000

Introduction

Various tissues of prion-infected animals contain a pathologically altered, disease-specific form of the normal 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 protease, PrP^{C} is destroyed, 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 PrP 27 – 30.

The Prionics®-Check LIA is a microplate-based immunoassay (ELISA) which detects protease-resistant PrP^{Sc} in brain tissue homogenates. Prionics®-Check LIA achieves its high precision and reliability through the unique properties of the buffer solutions and the high affinity of the two monoclonal antibodies directed against the prion protein. The microplate-based format enables complete automation for high throughput mass screening.

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 Annex III, chapter A to Regulation (EC) No 999/2001.

Principle of Test

After Sample Collection and Registration, samples are analyzed with the Prionics®-Check LIA Luminescence Immuno Assay. The Prionics®-Check LIA follows a five step protocol, consisting of Sample Preparation (Homogenization and Protease Digestion) and LIA-Assay (Preincubation, Capture and Detection). 240 homogenized samples can be analyzed in 4½ hours.

Samples are collected, registered, and a homogenate is prepared from a defined piece of brain tissue. Treatment with Proteinase K during Protease Digestion degrades PrP^{C} while PrP^{Sc} is reduced to the 27 – 30 kD fragment. The proteolytic reaction is stopped, and protease-resistant PrP^{Sc} is detected in the Prionics®-Check LIA-Assay.

The homogenates are incubated with the Detection Antibody. Protease-resistant PrP^{Sc} contained in the tissue homogenates binds to the Detection Antibody, which is an enzyme-labeled, monoclonal antibody. The reaction mixture is transferred to a microplate coated with the Capture Antibody which is also monoclonal. Complexes of Detection Antibody and PrP^{Sc} bind to the Capture Antibody. The microplate is washed and PrP^{Sc} bound by the two antibodies is detected by a chemiluminescence producing enzyme-substrate reaction.

Development, Distribution
Marketing & Sales

Manufacturing

PIERCE

Kit Contents

Shelf life of all components is 1 year after production. See kit label for actual expiry date.

Component 1
Homogenization Buffer (5x)
(5x concentrated, dilute before use) One bottle containing 570 ml of 5x concentrated Homogenization Buffer. Prepare 1x homogenization working solution by mixing 1 part Homogenization Buffer (5x) with 4 parts ultrapure water. Extra labels for the homogenization working solution are included in the kit.
Shelf life of the concentrated buffer: 12 months at 5 ± 3 °C
Shelf life of the homogenization working solution: 1 week at 5 ± 3 °C

Component 2
Digestion Buffer
(Ready-to-use) One vial containing 7 ml of Digestion Buffer.
Shelf life: 12 months at 5 ± 3 °C

Component 3
Digestion Plates (White Plates)
Five White Plates in which the protease digestion is performed.

Component 4
Sealing Film
20 pieces of Sealing Film to cover the Digestion, Preincubation and Capture Plates during incubations.

Component 5
Proteinase K
(Ready-to-use) One vial containing 7 ml of Proteinase K for the digestion of normal PrP^{C} during protease digestion.
Shelf life: 12 months at 5 ± 3 °C
Color of solution: blue
Cap color code: blue

Component 6
Digestion Stop
(Ready-to-use) One vial containing 7 ml of Proteinase K blocker to stop the proteolytic activity of the Proteinase K.
Shelf life: 12 months at 5 ± 3 °C
Cap color code: red

Component 7
Assay Buffer
(Ready-to-use) One vial containing 14 ml of Assay Buffer.
Shelf life: 12 months at 5 ± 3 °C
Adjust to room temperature 1h prior to use.

Component 8
Preincubation Plates (Blue Plates)
Five Blue Plates in which Preincubation and Detection Antibody incubations are performed.

Component 9
Negative Control
(Lyophilized) Five vials containing the lyophilized Negative Control.
One vial of Negative Control is reconstituted by adding first 200 µl ultrapure water and then 200 µl Assay Buffer (Component 7). Mix by vortexing thoroughly and inverting the tube several times. Reconstitute Control just prior to use.
Shelf life of the lyophilized Control: 12 months at 5 ± 3 °C
Shelf life of the reconstituted Control: 12 hours at room temperature
Cap color code: white

Component 10
Positive Control Low
(Lyophilized) Five vials containing the lyophilized functional Positive Control Low (recombinant bovine PrP).
One vial of functional Positive Control Low is reconstituted by adding first 200 µl ultrapure water and then 200 µl Assay Buffer (Component 7). Mix by vortexing thoroughly and inverting the tube several times. Reconstitute Control just prior to use.
Shelf life of the lyophilized Control: 12 months at 5 ± 3 °C
Shelf life of the reconstituted Control: 12 hours at room temperature
Cap color code: yellow

Component 11
Positive Control High
(Lyophilized) Five vials containing the lyophilized functional Positive Control High (recombinant bovine PrP).
One vial of functional Positive Control High is reconstituted by adding first 200 µl ultrapure water and then 200 µl Assay Buffer (Component 7). Mix by vortexing thoroughly and inverting the tube several times. Reconstitute Control just prior to use.
Shelf life of the lyophilized Control: 12 months at 5 ± 3 °C
Shelf life of the reconstituted Control:12 hours at room temperature
Cap color code: red

Component 12
Preincubation Buffer
(Ready-to-use) One vial containing 7 ml of Preincubation Buffer.
Shelf life: 12 months at 5 ± 3 °C
Cap color code: green

Component 13
Dilution Buffer
(Ready-to-use) One vial containing 140 ml of Dilution Buffer.
Shelf life: 12 months at 5 ± 3 °C

Component 14
Detection Antibody
(2500x concentrated, dilute before use) One vial containing 70 µl of the Detection Antibody. Dilute 1:2500 (see Appendix I) in Dilution Buffer (Component 13) just prior to use.
Shelf life of the concentrated Detection Antibody: 12 months at 5 ± 3 °C
Shelf life of the diluted Detection Antibody: 1 hour at room temperature, 12 hours at 5 ± 3 °C

Component 15
Capture Plates (Black Plates)
Five 96 well Black Plates coated with the Capture Antibody.
Shelf life: 12 months at 5 ± 3 °C

Component 16
Wash Buffer Powder
Five pouches containing the Wash Buffer Powder. Prepare wash buffer solution by dissolving the content of one pouch in 500 ml ultrapure water. Add 2.5 ml of Wash Buffer Detergent (Component 17). Extra labels for the wash buffer solution are contained in the kit.
Shelf life of the Wash Buffer Powder: 12 months at 5 ± 3 °C
Shelf life of the wash buffer solution: 1 week at room temperature

Component 17
Wash Buffer Detergent
One vial containing 15 ml of the Wash Buffer Detergent. Prepare wash buffer solution as described under Component 16.
Shelf life of the Wash Buffer Detergent: 12 months at 5 ± 3 °C

Component 18A, 18B
Chemiluminescent Substrates A and B
One bottle containing 31 ml of Chemiluminescent Substrate A and one bottle containing 31 ml of Chemiluminescent Substrate B. Prepare substrate working solution no longer than 30 min before use by mixing equal parts of Chemiluminescent Substrate A and B. The chemiluminescent substrate working solution is sensitive to light; store in dark.
Shelf life of components: 12 months at 5 ± 3 °C
Shelf life of substrate working solution: 30 min at room temperature if stored in dark

Additional Kit Contents:
- Extra labels for homogenization working solution (diluted version of Component 1)
- Extra labels for wash buffer solution (working solution prepared from Component 16 and Component 17)
- Package Insert

Additional Materials and Devices Required

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

General:
Laboratory equipment according to national safety regulations

- Ultrapure water: at least equivalent to Grade 3 water as defined by ISO 3696:1987 (E)

- Single channel pipette (0.5 – 10 µl)
- Single channel pipette (10 – 100 µl)
- Single channel pipette (100 – 1000 µl)
- Single channel pipette (1 – 5 ml)
- Multichannel pipette (5 – 50 µl)
- Multichannel pipette (50 – 300 µl)
- Pipette tips (as recommended by pipette manufacturer)
- Solution reservoirs
- 15 ml conical tubes
- 50 ml conical tubes

Homogenization:
- Disposable scalpels 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/FASTH 2 homogenization device (Consul AR S.A:, Product No: 80040, 82040, 80020) and Prypcon homogenization container (Consul AR S.A, Product No: 80300)

Digestion:
- Microplate incubator (reaching at least 50 °C)

Capture and Detection:
- ELISA plate shaker (500 rpm)
- ELISA plate washer (Multichannel, dispensation and reaspiration of 300 µl volumes)
- **Chemiluminescence ELISA plate reader** (Berthold, MPL2, Product No: 11220010)
  The RLU-factor of the Berthold MPL2 ELISA plate reader must be re-configured by trained service staff. Please contact your local distributor.

Analysis of Results:
- **Prionics®-Check LIA Analysis Software**

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 LIA 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 LIA.

Samples should be considered as potentially infectious and all items which contact 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 LIA, the following aspects should be considered:
- **The Test Procedure protocol must be strictly followed.**
- Pipette filter tips or separate pipettes for the different pipetting steps are strongly recommended. In addition, the accuracy of pipettes should be calibrated regularly.
- Pipette tips have to be changed for every pipetting step.
- Separate solution reservoirs must be used for each reagent.
- All solutions, except homogenates from the PrioCLIP™/Prypcon and Control Samples can be pipetted with multichannel pipettes.
- 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.
- Ultrapure water must be used for the test.
- Room temperature “RT” is defined as 22 ± 3 °C. A significant deviation of the de facto temperature from RT might influence the results of the assay.
- To mix and prepare Working Solutions where necessary, use only “single use” laboratory-grade plastic ware at all times. Do not use laboratory glass material. Detergents used during washing of laboratory glassware will negatively influence the assay procedure.
- Pipetting automates can be used provided that critical parameters described in the package insert – such as pipetted volumes, temperature and incubation times – are met.
- When the PrioGENIZER™ is used for homogenization, only program P0 PRIONICS TSE must be used for homogenization of brain tissue.

Pipetting schemes are shown in Appendix II of this Package Insert.

HOMOGENIZATION

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

Homogenization
- Transfer sample from the obex region to 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™ or the FASTH/MediFASTH/FASTH 2 homogenization device (45sec ± 5 sec, 20’000 ± 1’000 rpm)
- Store a 1 ml sample per homogenate in the 96-well sample Master Plate (see Appendix II) according to pipetting scheme.
- PrioCLIP™ and Prypcon homogenization containers of samples tested “TSE negative” may be washed for reuse (see PrioCLIP™ and Prypcon Wash Protocol, Appendix III)

PROTEASE DIGESTION

Preparatory Steps
- Allow Assay Buffer (Component 7) to adjust to RT for 1 hour (needed for Preincubation).
- Set the temperature of the microplate incubator to 47 °C approx. 1 hour prior to use.
- Prepare digestion solution (See Appendix I).
  Shelf life: 15 min at room temperature
- Add 50 µl of digestion solution to each well of the Digestion Plate according to pipetting scheme in Appendix II.

Protease Digestion
- Transfer 100 µl of each homogenate from the Master Plate into the Digestion Plate (see Appendix II). Afterwards, the Master Plate may be stored at -20 °C for 12 months. Mix the samples and the digestion solution by pipetting up and down. Visually control proper mixing of solutions.
- Cover the Digestion Plate with a Sealing Film.
- Digest for 60 ± 2 min at 47 ± 1 °C.
- Stop the reaction by adding 10 µl of Digestion Stop. Mix by pipetting up and down.
- After Digestion Stop has been added the Digestion Plate may be stored at -20 °C for up to 5 days.

PREINCUBATION

> **Attention**:
> It is critical for the performance of the test to adhere strictly to the incubation times indicated

Preparatory Steps
- Reconstitute Control Samples by adding 200 µl ultrapure water and 200 µl Assay Buffer (in this sequence). Mix by vortexing and inverting the tube several times.
- Dilute the Detection Antibody (Component 14; needed for Capture) according to Appendix I.

Preincubation
Controls
- Add 30 µl of Control Samples to the Preincubation Plate (Blue Plate) according to pipetting scheme (Appendix II).

Assay Buffer
- Add 15 µl of Assay Buffer to Preincubation Plate (Blue Plate) according to pipetting scheme (Sample wells only; see Appendix II).

Samples
- Transfer 15 µl of digested homogenates from the Digestion Plate to the Preincubation Plate, mix by up and down pipetting. Make sure to complete transfer and mixing of samples with Assay Buffer within **2 - 5 min**. Use a Lab Timer.

Preincubation Buffer
- After 2 - 5 min add 10 µl of Preincubation Buffer to **each** well of the Preincubation Plate, mix by pipetting up and down. Incubate samples for **2 min ± 15 s**. Use a Lab Timer.

CAPTURE

Detection Antibody Incubation
- Add 200 µl of Detection Antibody solution (Appendix I) to each well of the Preincubation Plate, cover the plate with Sealing Film and incubate the plate for 60 ± 5 min at RT (with shaking, 500 ± 50 rpm).

Capture
- Transfer 200 µl of each well from the Preincubation Plate to the Capture Plate and incubate for 90 ± 5 min at RT (with shaking, 500 ± 50 rpm).

DETECTION

General Remarks
- DO NOT USE hypochlorite containing disinfectants as they can interfere with test results.

Preparatory Steps
- Prepare wash buffer: Dissolve content of one pouch Wash Buffer Powder (component 16) in 500 ml ultrapure water. Add 2.5 ml of Wash Buffer Detergent (component 17) to 500 ml.
- Prime the ELISA washer with wash buffer solution.
- Prepare 10.4 ml Chemiluminescent Substrate working solution by mixing 5.2 ml of Chemiluminescent Substrates A and B each (components 18A and 18B). Use only disposable plastic ware to mix components.
  Shelf life of substrate working solution: 30 min at room temperature if stored in dark

Washing
- Wash the Capture Plate 4 times with 300 µl wash buffer solution per well (no incubation required). Clap plate several times to remove remaining liquid.
  Detailed protocols for different washers can be requested at info@prionics.com.

Luminescence Substrate Addition
- Add 100 µl per well of the Chemiluminescent Substrate working solution to the washed Plate.

Chemiluminescence Detection
- Put Capture Plate into plate reader and wait for 5 - 10 min. Then read out the Capture Plate.

INTERPRETATION OF RESULTS

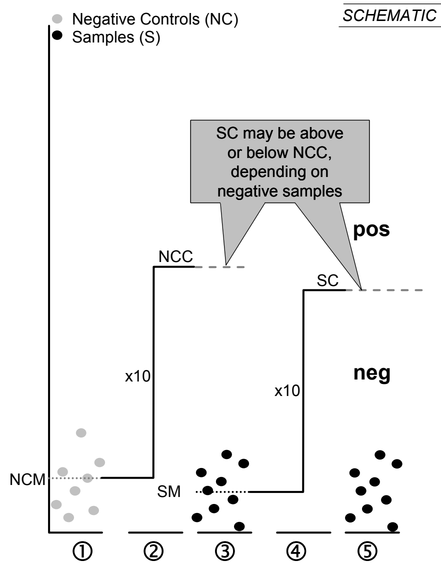
The values obtained by the plate luminometer are given as Relative Light Units and calculated by the Prionics®-Check LIA Analysis Software for identification of positive and negative results.
Alternatively, cutoff values may be calculated manually, following the same calculation protocol.
The Prionics®-Check LIA Analysis Software is provided free of charge and is an integral component of the Prionics®-Check LIA.

The cutoff is calculated in five steps for each plate:
This process allows to take both the general characteristics of the negative control and the individual characteristics of the particular plate into account.

- **Step 1:** The mean value of the Negative Controls (plate positions E1, E2, F1, F2, G1, G2, H1, H2) is calculated (NCM).
- **Step 2:** The mean value of the Negative Controls is multiplied by 10. This calculation defines the Negative Control Cutoff (NCC).
- **Step 3:** The mean of all sample values (plate positions A3 through H12) below NCC is calculated (SM).
- **Step 4:** The SM is multiplied by 10 to obtain the Sample Cutoff (SC).
- **Step 5:** Samples with values below the SC are identified negative. Samples with values above the SC are identified initially reactive. To ensure statistical representation, at least 8 samples have to be below the NCC. If less than 8 samples (per plate) are below the NCC in step 3, the NCC is taken as cutoff and samples above the NCC are identified initially reactive.

Initially reactive samples need to be retested in duplicate starting from their corresponding homogenates. If either one or both of the resulting repeat values are above the cutoff, the sample is identified positive. The result then needs to be indicated to the National Reference Laboratory and the remaining sample tissue must be sent in for confirmation. Additional national guidelines may apply.

Figure Cutoff Setting:



The test is validated for the following criteria (if they are not fulfilled, the results are inconclusive and the test must be repeated from the homogenate):
Average of Negative Controls: < 1'000 RLUs
Average of functional Positive Controls Low: > 5'000 RLUs
Average of functional Positive Controls High: > 100'000 RLUs
Sample Cutoff: < 10'000 RLUs

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.
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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

Table of Homogenization Buffer, Digestion Solution, and Dilution Buffer mixing ratios

Homogenization Working Solution
Mix indicated volumes of ultrapure water and Homogenization Buffer 5x concentrate to obtain the desired volume of homogenization working solution:

Vol. of homogenization working solution	Volume of Homogenization Buffer (5x)	Volume of ultrapure water
250 ml	50 ml	200 ml
500 ml	100 ml	400 ml
1000 ml	200 ml	800 ml
1500 ml	300 ml	1200 ml

Digestion Solution
Mix indicated volumes of Homogenization Working Solution, Digestion Buffer and Proteinase K (in this sequence) in a plastic tube (e.g., 50 ml reaction tube) just prior to use.
Shelf life of Digestion Solution: 15 min at room temperature

No. of plates	Vol. of digestion solution	Vol. of homogenization working solution	Vol. of Digestion Buffer	Vol. of Proteinase K
1	5 ml	3 ml	1 ml	1 ml
2	10 ml	6 ml	2 ml	2 ml
3	15 ml	9 ml	3 ml	3 ml
4	20 ml	12 ml	4 ml	4 ml
5	25 ml	15 ml	5 ml	5 ml

Diluted Detection Antibody
The table summarizes the volumes of Dilution Buffer needed for different numbers of Plates and the volume of Detection Antibody which has to be added to the Dilution Buffer for a 1:2500 dilution.

No. of plates	Volume of Dilution Buffer	Volume of Detection Antibody
1	25 ml	10 µl
2	50 ml	20 µl
3	75 ml	30 µl
4	100 ml	40 µl
5	125 ml	50 µl

If the test is run with less than 80 samples (one plate), reagent volumes need to be adapted accordingly.

Appendix II

Pipetting Schemes

Pipetting Scheme for Master Plate
For each homogenate, a 1 ml sample is pipetted into a 96-well 1.2 ml sample Master Plate which can be stored at -20 °C for up to 1 year. The position of each sample is indicated in the following scheme. This scheme has to be followed to assure the identification of the individual samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A			8	16	24	32	40	48	56	64	72	80
B			7	15	23	31	39	47	55	63	71	79
C			6	14	22	30	38	46	54	62	70	78
D			5	13	21	29	37	45	53	61	69	77
E			4	12	20	28	36	44	52	60	68	76
F			3	11	19	27	35	43	51	59	67	75
G			2	10	18	26	34	42	50	58	66	74
H			1	9	17	25	33	41	49	57	65	73

Pipetting Scheme for Digestion Plate (Component 3, White Plate)
The prepared homogenates (samples) are transferred from the Master Plate to the Digestion Plate according to the following pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A			8	16	24	32	40	48	56	64	72	80
B			7	15	23	31	39	47	55	63	71	79
C			6	14	22	30	38	46	54	62	70	78
D			5	13	21	29	37	45	53	61	69	77
E			4	12	20	28	36	44	52	60	68	76
F			3	11	19	27	35	43	51	59	67	75
G			2	10	18	26	34	42	50	58	66	74
H			1	9	17	25	33	41	49	57	65	73

Pipetting Scheme for each single Preincubation Plate (Component 8, Blue Plate)
The digested samples from the Digestion Plate and the reconstituted Controls (functional Positive High, functional Positive Low, Negative) are transferred to the Preincubation Plate according to the following pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive Control High		8	16	24	32	40	48	56	64	72	80
B	Positive Control High		7	15	23	31	39	47	55	63	71	79
C	Positive Control Low		6	14	22	30	38	46	54	62	70	78
D	Positive Control Low		5	13	21	29	37	45	53	61	69	77
E	Negative Control		4	12	20	28	36	44	52	60	68	76
F	Negative Control		3	11	19	27	35	43	51	59	67	75
G	Negative Control		2	10	18	26	34	42	50	58	66	74
H	Negative Control		1	9	17	25	33	41	49	57	65	73

Pipetting Scheme for each single Capture Plate (Component 15, Black Plate)
The samples and controls from the Preincubation Plate are transferred to the Capture Plate according to the following pipetting scheme

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive Control High		8	16	24	32	40	48	56	64	72	80
B	Positive Control High		7	15	23	31	39	47	55	63	71	79
C	Positive Control Low		6	14	22	30	38	46	54	62	70	78
D	Positive Control Low		5	13	21	29	37	45	53	61	69	77
E	Negative Control		4	12	20	28	36	44	52	60	68	76
F	Negative Control		3	11	19	27	35	43	51	59	67	75
G	Negative Control		2	10	18	26	34	42	50	58	66	74
H	Negative Control		1	9	17	25	33	41	49	57	65	73

Appendix III

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 used again 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 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 IV

Potential Problems and Solutions
The Prionics®-Check LIA protocol must be strictly followed to avoid losses in test quality. Potential problems in test performance and possible solutions are listed below:

Problem
No signal
Potential causes
- Assay buffer omitted
- Digestion Plate or Preincubation Plate instead of Capture Plate used during Detection Antibody incubation
- Detection Antibody omitted or used at wrong dilution
- Luminescence substrate working solution wrongly prepared
- Functional Positive Control Samples omitted
- Contamination of reagents or water

Potential solutions
- Repeat test and strictly follow the instructions
- Change reagents and check quality of water

Problem
Generally low signals compared to control values
Potential causes
- Assay Buffer omitted
- Wrong concentration of Detection Antibody
- Inappropriate addition of Preincubation Buffer
- Contamination of reagents or water

Potential solutions
- Repeat test and strictly follow the instructions
- Change reagents and check quality of water

Problem
Generally high signals compared to control values
Potential causes
- Inappropriate washing
- Prolonged incubation times
- Wrong concentration of Detection Antibody
- Contamination of reagents or water

Potential solutions
- Repeat test and strictly follow the instructions
- Change reagents and check quality of water

Problem
Initially reactives negative after retesting.
The high sensitivity of the test may cause a small number of samples to be initially reactive, but negative after retesting. Initially reactive values are typically just above the cutoff, ranging between 100% and 250% of the cutoff value.
Potential cause
- Incomplete digestion
- Incomplete digestion can be visualized with the Prionics®-Check WESTERN

Potential solution
- Make sure the digestion step is carried out at the appropriate temperature with the appropriate amount of Proteinase K

Potential cause
- Detection artifacts
- Initially reactives caused by detection artifacts can be identified by re-analyzing the digested samples with the Prionics®-Check LIA

Potential solution
- Make sure to pipet solutions with care and not to carry over functional Positive Control material into sample wells
- Store pipet tips in clean boxes

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: R36/37/38 Irritating to eyes, respiratory system and skin.
S2 Keep out of the reach of children.
S46 If swallowed, seek medical advice immediately and show this container or label.

Component 2
Digestion Buffer
Hazard Code: R22 Harmful if swallowed
R36/38 Irritating to eyes and skin.
S2 Keep out of the reach of children.
S46 If swallowed, seek medical advice immediately and show this container or label.

Component 3
Digestion Plates (White Plates)
Hazard Code: This product is not classified according to EU regulations.

Component 4
Sealing Film
Hazard Code: This product is not classified according to EU regulations.

Component 5
Proteinase K
Hazard Code: R36/37/38 Irritating to eyes, respiratory system and skin.
S2 Keep out of the reach of children.
S46 If swallowed, seek medical advice immediately and show this container or label.

Component 6
Digestion Stop
Hazard Code: This product is not classified according to EU regulations.

Component 7
Assay Buffer
Hazard Code: R22 Harmful if swallowed
R36/38 Irritating to eyes and skin.
S2 Keep out of the reach of children.
S46 If swallowed, seek medical advice immediately and show this container or label.

Component 8
Preincubation Plates (Blue Plates)
Hazard Code: This product is not classified according to EU regulations.

Component 9
Negative Control
Hazard Code: This product is not classified according to EU regulations.

Component 10
Positive Control Low
Hazard Code: This product is not classified according to EU regulations.

Component 11
Positive Control High
Hazard Code: This product is not classified according to EU regulations.

Component 12
Preincubation Buffer
Hazard Code: R8 Contact with combustible material may cause fire.
S2 Keep out of the reach of children

Component 13
Dilution Buffer
Hazard Code: This product is not classified according to EU regulations.

Component 14
Detection Antibody
Hazard Code: This product is not classified according to EU regulations.

Component 15
Capture Plates (Black Plates)
Hazard Code: This product is not classified according to EU regulations.

Component 16
Wash Buffer Powder
Hazard Code: This product is not classified according to EU regulations.

Component 17
Wash Buffer Detergent
Hazard Code: This product is not classified according to EU regulations.

Component 18A
Chemiluminescent Substrate A
Hazard Code: R36/37/38 Irritating to eyes, respiratory system and skin.
S2 Keep out of the reach of children.
S46 If swallowed, seek medical advice immediately and show this container or label.

Component 18B
Chemiluminescent Substrate B
Hazard Code: This product is not classified according to EU regulations.

Appendix VI

Contact and Distributors
Principle Contact:

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Kit Components are manufactured for Prionics AG by Pierce Biotechnology, Rockford, Illinois USA, a subsidiary of Fisher Scientific.
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For our distribution network, please refer to www.prionics.com

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