PrioSTRIP BSE Kit

Test for in vitro detection of TSE-related PrPsc

Catalog Number 30000

Pub. No. MAN0013791 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

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

The producer of the rapid tests must have a quality assurance system in place agreed by the European Union Reference Laboratory, which ensures that the test performance does not change. The producer must provide the test protocol to the European Union 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 European Union Reference Laboratory (EURL) and provided that the European Union 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 prion-infected animals contain a pathologically altered, disease-specific form of the normal prion protein (PrP). The altered prion protein is called 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 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 PrP 27–30.

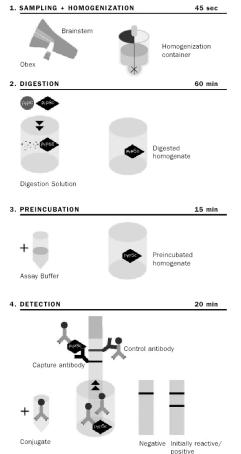
The Applied Biosystems[™] PrioSTRIP BSE Kit is an immunochromatographic assay which detects PrP^{Sc} in brain tissue homogenates. PrioSTRIP BSE Kit achieves its high precision and reliability through the unique properties of the buffer solutions and the high affinities of the two monoclonal antibodies directed against the prion protein.

Test principle

After Sample Collection and Registration, samples are analyzed with the PrioSTRIP BSE Kit immunochromatographic assay. The PrioSTRIP BSE Kit follows a four step protocol, consisting of Homogenization, Protease Digestion, Preincubation and Detection. 470 homogenized samples can be analyzed in less than 2½ hours.

Samples are collected, registered, and a homogenate is prepared from a defined piece of tissue (medulla oblongata) of the obex region in the brain stem. 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 PrioSTRIP BSE Kit assay.

Digested homogenates are incubated with the antibody Conjugate. PrP^{sc} present in the homogenates binds to the Conjugate, which is a latex bead-labeled monoclonal antibody. By dipping the PrioSTRIP into the sample-Conjugate-mixture, the flow through the membrane is started. PrP-Conjugate complexes are retained at the test line by the second (capture) antibody. Uncomplexed Conjugate is bound at the control line serving as a control for the proper performance of the immunochromatographic assay.



Kit components (Continued on the next page)

Kit for 470 samples. Store kit at $5\pm3^{\circ}$ C until expiry date. See kit label for actual expiry date. The shelf life of diluted, opened or reconstituted components is noted below, when appropriate.

Component	Description
1: Homogenization Buffer (5x) (Cap color code: purple)	5x concentrated, dilute before use. Three bottles containing 220 mL of 5x concentrated Homogenization Buffer each. Prepare 1x homogenization working solution by mixing 1 part Homogenization Buffer (5x) with 4 parts purified water. Shelf life of the 1x homogenization working solution: 1 week at 5±3°C.
2: Digestion Buffer (Cap color code: white)	Ready-to-use. One vial containing 7.5 mL of Digestion Buffer.
3: Digestion Plates (Colorless Plates)	Five Colorless Plates in which the protease digestion is performed.
4: Sealing Film	10 pieces of Sealing Film to cover the Digestion Plates during incubations.
5: Proteinase K (Cap color code: yellow)	Ready-to-use. One vial containing 7.5 mL of Proteinase K for the digestion of normal PrP^{c} during protease digestion.
6: Digestion Stop (Cap color code: red)	Ready-to-use. One vial containing 7.5 mL of Proteinase K blocker to stop the proteolytic activity of the Proteinase K.
7: Assay Buffer (Color of solution: green)	Ready-to-use. One bottle containing 80 mL Assay Buffer. Place at 22±3°C at least 2 hours prior to use. Visually check for precipitates before use.
8: Test Plates (White Plates)	Five white flat-bottom microplates in which PrioSTRIP detection is performed.
9: Conjugate Buffer (Cap color code: blue)	Ready-to-use. One bottle containing 50 mL of Conjugate Buffer.
10: Conjugate	Lyophilized. Five vials containing the lyophilized blue Conjugate. The Conjugate is reconstituted by adding 9 mL Conjugate Buffer (Component 9). Mix immediately by vortexing vigorously for at least 15 seconds upon addition of conjugate buffer and just prior to each use. Shelf life of the reconstituted Conjugate: 1 week at 5±3°C.



Component	Description
11: PrioSTRIP Combs	Ten pouches containing six combs each. With each PrioSTRIP comb 8 samples can be analyzed. Return unused PrioSTRIP combs to their pouch, close pouch and store at 5±3°C. Adjust to 22±3°C for at least 30 minutes prior to opening the pouch. Shelf life in opened pouches: 2 weeks at 5±3°C.
12: Positive Control (Cap color code: red)	Lyophilized. Five vials containing the lyophilized functional Positive Control. One vial of functional Positive Control is reconstituted by adding first 200 μ L purified water and then 200 μ L Assay Buffer (Component 7). Mix by vortexing thoroughly and inverting the tube several times. Shelf life of the reconstituted Control: 12 hours at 22±3°C.
13: Negative Control (Cap color code: white)	Lyophilized. Five vials containing the lyophilized Negative Control. One vial of Negative Control is reconstituted by adding first 200 μ L purified water and then 200 μ L Assay Buffer (Component 7). Mix by vortexing thoroughly and inverting the tube several times. Shelf life of the reconstituted Control: 12 hours at 22±3°C.
Additional kit contents	 Five PrioSTRIP Visual Interpretation Sheets. Make photocopies if necessary. Package Insert PrioSTRIP lot calibration sheet

Additional material required

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

Use	Description
General Homoge-	 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 (10–100 μL) Single channel pipette (100–1,000 μL) Single channel pipette (1–5 mL) Multichannel pipette (50–300 μL) Pipette tips (as recommended by pipette manufacturer) Solution reservoirs 15 mL conical tubes Vortex Cutting tool and forceps
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[™] Container Box (Cat. No. PR10010). or FASTH/FASTH 2/MediFASTH homogenization device (Syntec International) and Prypcon homogenization container (Syntec International)
Digestion	 Microplate incubator (reaching at least 50°C)
Preincubation	 If required, the functional PrioSTRIP BSE Positive Control (Cat. No. 3000012) and PrioSTRIP BSE Negative Control (Cat. No. 3000013) can be ordered separately with indication of kit lot number. Note that only Controls with the same lot number as the Controls in the kit can be used.
Analysis of results	 PrioSCAN[™] Package (device and software; Cat. No: 30900)

Test procedure

Precautions

- National Safety Regulations must be strictly followed.
- The PrioSTRIP 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 PrioSTRIP BSE Kit.
- Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

Notes

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

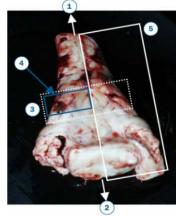
- The Test Procedure protocol must be strictly followed.
- Pipette tips have to be changed for every pipetting step.
- The use of either pipette filter tips or separate pipettes for the different pipetting steps is strongly recommended. In addition, the accuracy of pipettes should be calibrated regularly.
- Separate solution reservoirs must be used for each reagent.
- All solutions, except those for preparation of digestion solution, homogenates from the homogenization containers 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.
- Purified water must be used for the test.
- Non-disposable cutting tools and forceps must be decontaminated according to guidelines enforced by national authorities.

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 and weigh the sample to ensure the correct amount.

Sampling and laboratory testing must follow the Regulation (EC) No. 999/2001 Chapter C which refers in terms of collection of samples to the latest edition of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 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.



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

Medulla oblongata (obex region)

The tissue sample is an approx. 8 cm-long piece of brain stem / 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 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 from the obex region) and homogenize sample using the PrioGENIZER[™] or FASTH/FASTH2/MediFASTH homogenization device (45±5 seconds, 20,000±1,000 rpm).
- Store a 1 mL sample per homogenate in a 96-well sample Master Plate (omit wells A1 and B1, Appendix B).
- PrioCLIP[™] and Prypcon homogenization containers of samples tested "TSE negative" may be washed for reuse (see PrioCLIP[™]/Prypcon Wash Protocol, Appendix C).

Protease digestion

Preparatory steps

- Set the temperature of the microplate incubator to $47\pm1^{\circ}C$ approx. 1 hour prior to use.
- Prepare digestion solution (See Appendix A). Shelf life: 15 minutes at 22±3°C.
- Add 50 μL of digestion solution to each well of the Digestion Plate (Component 3, Colorless Plate). Omit wells A1 and B1.

Protease digestion

- Transfer 100 μ L (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 (Appendix B). Afterwards, the Master Plate may be covered and stored at 5±3°C for up to 8 hours or at -20°C to -80°C for up to 12 months. Mix the samples and the digestion solution by pipetting up and down at least three times.
- Cover the Digestion Plate with a Sealing Film (Component 4).
- Digest for 60±2 minutes at 47±1°C.
- Stop the reaction by adding 10 μL of Digestion Stop (Component 6). Mix by pipetting up and down at least three times.
- After Digestion Stop has been added the Digestion Plate may be covered with a Sealing Film and stored at -20°C to -80°C for up to 5 days.

Preincubation

Preparatory steps

- Place Assay Buffer (Component 7) at 22±3°C for at least 2 hours prior to use.
- Reconstitute the functional Positive Control (Component 12) by adding first 200 μ L water and then 200 μ L Assay Buffer (Component 7, green). Mix by vortexing and inverting the tube several times.
- Reconstitute the Negative Control (Component 13) by adding first 200 μL water and then 200 μL Assay Buffer (Component 7, green). Mix by vortexing and inverting the tube several times.
- If required, the functional Positive and Negative Controls can be ordered separately (see "Additional material required").

Preincubation

- Add 150 μL of Assay Buffer (mix thoroughly before use) to the digested homogenate in the Digestion Plate and mix by pipetting up and down at least three times.
- Incubate at 22±3°C for 15±1 min.
- Add first 12 μ L of the reconstituted Negative Control to well B1 and then 12 μ L of the reconstituted functional Positive Control to well A1 of the Test Plate (Component 8, White Plate, Appendix B).
- Transfer 12 µL of the preincubated sample (mix again by pipetting up and down at least five times) from the Digestion Plate to the Test Plate. Visually control proper transfer (Appendix B).

Detection

Preparatory steps

- Reconstitute the lyophilized Conjugate (Component 10) by adding 9 mL Conjugate Buffer (Component 9). Mix immediately by vortexing vigorously for at least 15 seconds upon addition of Conjugate Buffer and just prior to each use.
- Place pouches containing the PrioSTRIP combs (Component 11) at 22±3°C, at least 30 minutes prior to opening.

Detection

- Add 80 µL of the reconstituted Conjugate to each well containing sample or control of the Test Plate using a multichannel pipette. Mix by slowly pipetting up and down twice.
- Lower the PrioSTRIP combs into the sample mixture. Place the combs in the Test Plate such that strip A of the PrioSTRIP comb is in a well of row A and strip B in a well of row B etc.
- Leave PrioSTRIP in the wells for 20±1 minutes at 22±3°C.
- Interpret and scan results within 10 minutes.

Interpretation of results

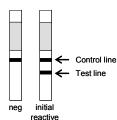
Visual interpretation

Visual interpretation requires two people (readers) who individually interpret each result. In all samples the control line must appear. A PrioSTRIP **Visual Interpretation Sheet** is supplied for each PrioSTRIP Test Plate. Carefully follow the instructions on the Visual Interpretation Sheet.

The sample is:

- Negative if only the Control line is visible.
 An initial reactive if both the Control line
- and the Test line (1–2 mm below the Control line) are visible.

• Invalid if no lines or only Test line appears. The readers then compare their results. Positive and Negative Controls must show a correct result. If not, the entire plate is invalid and has to be retested starting from the corresponding homogenates.



Results

- All samples found to be an **initial reactive** by one or both readers need to be retested in duplicate starting from their corresponding homogenates. The two outcomes resulting from retesting an initially reactive sample are then individually interpreted by the two readers. The readers compare their results and in case one or both readers interpret the result as positive or invalid, the result needs to be indicated to the National Reference Laboratory. If both readers interpret the result as negative, the sample is negative.
- A sample found to be **invalid** by one or both readers needs to be retested (single) starting from the corresponding homogenate. The outcome resulting from retesting an invalid sample is individually interpreted by the two readers. The two readers compare their results.
 - If both readers interpret the outcome as negative, the sample is negative.
 - If one or both readers interpret the outcome as invalid the sample should be referred to the National Reference Laboratory.
 - If one or both readers interpret the outcome as initial reactive, the sample should be regarded as initial reactive and retested in duplicate starting from the corresponding homogenate.
- Additional national guidelines may apply.

Interpretation with the PrioSCAN"

The PrioSCAN[™] converts the blue lines on the strips into digital data. The values obtained with the PrioSCAN[™] are given as Relative Density Units (RDU). The cutoff is lot-dependent and provided with each new lot, encoded on the lot calibration sheet.

- The sample is:
 - **Negative**, if the value of the test line is below cutoff and the control line is present.
 - **Initial reactive**, if the value of the test line is above cutoff and the control line is present.
 - Invalid, if no control line is present.

If the Negative Control or the functional Positive Control or both do not show the correct result, the plate is invalid and all samples on the plate have to be retested from the corresponding homogenates. **Results**

- All samples found to be **initial reactive** need to be retested in duplicate starting from their corresponding homogenates. In case one or both results are detected as positive or invalid, the result needs to be indicated to the National Reference Laboratory.
- All samples found to be **invalid** need to be retested (single) starting from their corresponding homogenates.
 - If the result is interpreted as negative the sample is negative.
 - If the result is interpreted as invalid the result needs to be indicated to the National Reference Laboratory.
 - If the result is interpreted as initial reactive, the sample should be regarded as initial reactive and retested in duplicate starting from the corresponding homogenate.

Additional national guidelines may apply.

For visual and automated interpretation

Samples and the corresponding tissue giving positive or invalid rapid test results should be sent to the NRL for confirmation.

If visual interpretation is used, laboratories testing under EU Regulation 999/2001 must create legible records of all combs (for example electronically by scanning or taking a digital photo).

Appendix A – Tables for preparation of solutions

Homogenization working solution

Mix indicated volumes of purified 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 $5\pm3^{\circ}$ 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
1,000 mL	=	200 mL	+	800 mL
1,500 mL	=	300 mL	+	1,200 mL

Digestion solution

Mix indicated volumes of homogenization working solution, Digestion Buffer (Component 2) and Proteinase K (Component 5) (in this sequence) in a single-use plastic tube (e.g., 15 mL reaction tube) just prior to use. Shelf life of digestion solution: 15±1 minutes at 22±3°C

Shen m	Shell life of digestion solution. 15±1 limitutes at 22±5 C.								
No. of	Vol. of		Vol. of	Vol. of			Vol. of		
plates	digestion	=	homogenization	+	Digestion Buffer	+	Proteinase K		
plates	solution		working solution		(Component 2)		(Component 5)		
1	6 mL	=	3.6 mL	+	1.2 mL	+	1.2 mL		
2	12 mL	=	7.2 mL	+	2.4 mL	+	2.4 mL		
3	18 mL	=	10.8 mL	+	3.6 mL	+	3.6 mL		
4	24 mL	=	14.4 mL	+	4.8 mL	+	4.8 mL		
5	30 mL	=	18 mL	+	6 mL	+	6 mL		

Appendix B - Recommended pipetting schemes Master Plate and Digestion Plate (Component 3, Colorless Plate)

	1	2	3	4	5	6	7	8	9	10	11	12
Α		Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	Sample 47	Sample 55	Sample 63	Sample 71	Sample 79	Sample 87
В		Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	Sample 48	Sample 56	Sample 64	Sample 72	Sample 80	Sample 88
С	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	1	9	17	25	33	41	49	57	65	73	81	89
D	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	2	10	18	26	34	42	50	58	66	74	82	90
Е	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	3	11	19	27	35	43	51	59	67	75	83	91
F	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36	Sample 44	Sample 52	Sample 60	Sample 68	Sample 76	Sample 84	Sample 92
G	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	5	13	21	29	37	45	53	61	69	77	85	93
Н	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	6	14	22	30	38	46	54	62	70	78	86	94

Test Plate (Component 8, White Plate)

	1	2	3	4	5	6	7	8	9	10	11	12
Α	+	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	Sample 47	Sample 55	Sample 63	Sample 71	Sample 79	Sample 87
В	-	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	Sample 48	Sample 56	Sample 64	Sample 72	Sample 80	Sample 88
С	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	1	9	17	25	33	41	49	57	65	73	81	89
D	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	2	10	18	26	34	42	50	58	66	74	82	90
Е	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	3	11	19	27	35	43	51	59	67	75	83	91
F	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	4	12	20	28	36	44	52	60	68	76	84	92
G	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	5	13	21	29	37	45	53	61	69	77	85	93
Н	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	6	14	22	30	38	46	54	62	70	78	86	94

+ Positive Control; - Negative Control

Appendix C – PrioCLIP[™]/Prypcon wash protocol

General instructions

Sample traceability

PrioCLIP[™]/Prypcon homogenization containers must be labeled with sample number-using e.g. 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.

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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 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 reuse.

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 D - 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-acdptse-risk-management-subgroup-formerly-tse-working-group.

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- 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

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Revision history of Pub. No. MAN0013791 (English)

Rev.	Date Description							
A.0	8 September 2017	New document. Converted the legacy document [PrioSTRIP_Pl_v4.0_e_220512.doc] to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos.						

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