TeSeE™ Western Blot

Σ 32 REF 3551169

REAGENTS FOR *IN VITRO* CONFIRMATION OF SUSPECTED TSE POSITIVE SAMPLES

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

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BIO-RAD

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1 - GENERAL INFORMATION

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

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

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

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

2. To confirm the prevalence of infection with one of the TSE associated diseases (BSE, scrapie, CWD) in the context of an epidemiological survey in a low prevalence country;
3. To estimate prevalence of infection to facilitate risk analysis (e.g. surveys, implementation of disease control measures) and to assist the demonstration of the efficiency of eradication policies.

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

2 - ASSAY PRINCIPLE

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

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

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

3 - COMPOSITION OF THE KIT

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

[1] These reagents contain 0.1 % of ProClin™ 300 (preservative).

4 - SAMPLES

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

Bovine: purification of PrP^{res} is performed on samples from Central Nervous System (CNS). Since distribution of PrP^{res} is heterogeneous in central nervous system, obex area from brainstem must be preferably sampled for optimal detection.
Sampling syringe (Ref.: 3551175) allows easy and rapid sampling of obex area in a secure way. Please refer to sampling protocol provided with sample syringes for detailed instructions on good sampling procedure.

Small ruminants: purification of PrP^{res} is performed on samples from Central Nervous System (CNS).
Samples are cut and weighed individually.

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

5 - ASSAY PROCEDURE WITH MINI BLOT™ GEL

5.1 - ADDITIONAL REAGENTS AND MATERIAL REQUIRED
5.1.1 - REAGENTS AND DISPOSABLES

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

Sample purification

Laemmli sample buffer	30 ml	Bio-Rad, cat. Nr. 1610737
2-Mercaptoethanol	25 ml	Bio-Rad, cat. Nr. 1610710
SDS	100 g	Bio-Rad, cat. Nr. 1610301
Calibration syringes	200	Bio-Rad, cat. Nr. 3551174

Gel electrophoresis

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

Immunoblotting

Ethanol (Normapur)	1L	VWR, cat. Nr. 20821-296
Tris/CAPS (transfer buffer) (10x)	1L	Bio-Rad, cat. Nr. 1610778
Filter paper (transfer paper for Mini Blot™ handcast gels)	50 sheets	Bio-Rad, cat. Nr. 1703932

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

5.1.2 - EQUIPMENT

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

Sample purification

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

Gel electrophoresis

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

Transfer

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

Immunoblotting

Western Processor base	Bio-Rad, Discontinued
Western Processor Mini Blot™ kit	Bio-Rad, Discontinued

5.2 - PREPARATION OF REAGENTS

5.2.1 - SAMPLE PURIFICATION

• Proteinase K

Solution of proteinase K diluted in reagent A:

- ▶ 1 ml Reagent A
- ▶ 20 µl Proteinase K

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

• Laemmli solution

Solution of SDS + 2-Mercaptoethanol + Laemmli sample buffer:

- ▶ 0.6 g SDS
- ▶ 1.5 ml 2-Mercaptoethanol

Mix by inverting.

- ▶ 28.5 ml Laemmli sample buffer

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

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

5.2.2 - ELECTROPHORESIS

• Hand cast discontinuous acrylamide gel

The gel must be 1.5 mm thickness.

Using the Mini Blot™ casting module, the resolving gel (13.5% acrylamide, pH 8.8) is cast first, once the resolving gel is polymerized the stacking gel is added (3% acrylamide, pH 6.8).

Resolving gel (1 gel)

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

Mix by inverting.

- ▶ 43 µl 10% Ammonium persulfate (3)
- ▶ 9 µl TEMED

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

Stacking gel (1 gel)

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

Mix by inverting.
Gently pour the stacking gel onto the resolving gel and retain the residual solution as a control of polymerization. Position the comb, taking care not to trap any bubble in the well positions.
Let the gel polymerize for 5-10 minutes at room temperature (+18°C to +30°C). Check the residual solution is polymerized.

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

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

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

(2) Solution of 50% Sucrose

- ▶ 25 g Sucrose
- ▶ to 50 ml Distilled water

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

(3) Solution of 10% Ammonium persulfate

- ▶ 5 g Ammonium persulfate
- ▶ to 50 ml Distilled water

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

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

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

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

(5) Solution of 0.5 M Tris-HCl buffer, pH 6.8 / SDS

- ▶ 0.2 g SDS
- ▶ 50 ml 0.5 M Tris-HCl buffer pH 6.8

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

(6) Solution of 1% Bromophenol Blue

- ▶ 0.5 g Bromophenol Blue
- ▶ 50 ml Distilled water

Bromophenol Blue solution can be stored at room temperature (+18°C to +30°C) for 6 months.

(7) Solution of 3% acrylamide

- ▶ 3.8 ml Acrylamide 40%, 29:1
- ▶ 10 ml 0.5 M Tris-HCl buffer pH 6.8 / SDS (5)
- ▶ 6 ml Sucrose 50% (2)
- ▶ 500 µl Bromophenol Blue 1% (6)
- ▶ to 50 ml Distilled water

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

• Kaleidoscope™ prestained standard

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

• MagicMark™ XP Western Standard

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

• Mini Blot™ migration buffer

Solution of Tris-Glycine-SDS (1x).
Prepare a 1/10 dilution. **1 L of diluted buffer is required for 1 tank:**

- ▶ 900 ml Distilled water
- ▶ 100 ml Tris-Glycine-SDS buffer (10x)

Homogenize. Solution can not be stored.

5.2.3 - PROTEIN TRANSFER

• Transfer buffer

Solution of Tris/Caps-Ethanol 15%. 2.5 L is required for 1 transfer tank.

- ▶ 750 ml Distilled water
- ▶ 150 ml Pure ethanol
- ▶ 100 ml Tris/CAPS (10x)

Homogenize. Solution can not be stored.

5.2.4 - IMMUNOBLOTTING

• Wash solution 1

Solution of PBS (1x) + 0.1% Tween® 20. **Approximately 500 ml is required for the complete process of 1 membrane.**

- ▶ 900 ml Distilled water
- ▶ 100 ml PBS (10x)
- ▶ 1 ml Tween® 20

Thoroughly homogenize. Solution can be stored at +2°C to +8°C, overnight.

• Wash solution 2

Solution of PBS (1x). **Approximately 100 ml is required for the complete process of 1 membrane**.

- ▶ 900 ml Distilled water
- ▶ 100 ml PBS (10x)

Solution can be stored at room temperature (+18°C to +30°C) overnight.

• Blocking solution

During the transfer step, dilute the blocking solution (Bl) 1/10 in Wash solution 1. **20 ml of diluted blocking solution (1x) is required for 1 membrane**.

- ▶ 18 ml Wash solution 1
- ▶ 2 ml Blocking solution (10x)

Homogenize by tube inverting.

• Diluted primary antibody

Just prior to use, dilute the primary antibody 1/10 in Wash solution 1.
15 ml of diluted primary antibody is required for 1 membrane.

- ▶ 13.5 ml Wash solution 1
- ▶ 1.5 ml Primary antibody (10x)

Homogenize by tube inverting.

• Diluted secondary antibody (conjugate)

Just before use, dilute the secondary antibody 1/10 in Wash solution 1.
20 ml of diluted conjugate is required for 1 membrane.

- ▶ 18 ml Wash solution 1
- ▶ 2 ml Secondary antibody (10x)

Homogenize by tube inverting.

• ECL

Substrate (ECL) must be prepared just before use. **1 ml of substrate is required for 1 membrane**.

- ▶ 0.5 ml Reagent 1
- ▶ 0.5 ml Reagent 2

Homogenize the solution.

• Development solution

- ▶ 800 ml Distilled water
- ▶ 200 ml Development product

Solution can be stored at room temperature (+18°C to +30°C), in a darkroom for 15 days maximum.

• Fixative solution

- ▶ 800 ml Distilled water
- ▶ 200 ml Fixative product

Solution can be stored at room temperature (+18°C to +30°C), in a darkroom for 15 days maximum.

5.3 - SAMPLE PURIFICATION

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

Sampling

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

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

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

Sample grinding

Place the tubes in the crown of the homogenizer.

Perform one agitation cycle with the following instrument parameters.

	Ribolyser®		**TeSeE™ PRECESS 48™ or TeSeE™ PRECESS 24™**	
	Nervous tissues	Peripheral tissues	Nervous tissues	Peripheral tissues
Time (sec.)	45	2 x 45[1]	-	-
Speed	6.5	6.5	-	-
Program	-	-	Program 1	Program 2

When grinding is insufficient, another 1 or 2 agitation cycles can be performed[2].

[1] [2] Wait a 5 minutes pause between the 2 agitation cycles.

Sample calibration

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

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

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

- At room temperature (+18°C to +30°C) for 15 hours.

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

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

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

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

Concentration of the PrP^{res} by centrifugation
Centrifuge the tubes for 7 minutes at 15000 g at 20°C.

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

5.4 - ELECTROPHORESIS

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

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

Sample loading
Heat the samples for 4 minutes at 100 °C ± 5°C just prior to loading 15 µl/well. Load 15 µl of the diluted Kaleidoscope™ prestained standard and 15 µl of the diluted MagicMark™ XP (see paragraph 5.2.2).
Note: In case several gels are run at the same time, make sure that you stagger the loading of controls into different lanes for easy identification.
Differential migration of the samples
Run the gel at room temperature (+18°C to +30°C) for 90 minutes at 150 V. The blue line must be out of the gel.

5.5 - PROTEIN TRANSFER

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

Protein transfer preparation
Cut the membrane to the gel dimensions. Always use forceps when handling the membrane.
Immerse the membrane in pure ethanol for 15 seconds, rinse in distilled water for 5 minutes, then for 10 minutes in the transfer buffer.
Carefully remove the gel from the glass plates and let it equilibrate for 10 minutes in the transfer buffer.

Gel sandwich preparation
Soak filter paper and fibre pads in the transfer buffer.
Open the transfer cassette, with transparent side on the left. Respectively place on the transparent side a fiber pad, a filter paper, the membrane* and the gel*.
Complete with a filter paper then a fibre pad and close the cassette.
Immerse it in the transfer tank previously filled to the indicated limit with transfer buffer.
*Remove any air bubbles which may have formed.
Note: In case several membranes are processed at the same time, label each membrane in the corner.

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

5.6 - IMMUNOBLOTTING

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

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

c) Eliminate the blocking solution and incubate the membrane in diluted **primary antibody** (see paragraph 5.2.4) for 30 minutes at room temperature (+18°C to +30°C) under medium agitation.
15 ml of diluted primary antibody is required for 1 membrane.

d) Eliminate the primary antibody solution and using Wash solution 1, briefly rinse the membrane, then wash twice for respectively 5 and 10 minutes, under fast agitation.
50 ml of Wash solution 1 is required for each cycle and for 1 membrane.

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

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

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

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

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

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

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

l) Wash with distilled water and let the film dry.

6 - ASSAY PROCEDURE WITH CRITERION™ XT GEL

6.1 - ADDITIONAL REAGENTS AND MATERIAL REQUIRED

6.1.1 - REAGENTS AND DISPOSABLES

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

Sample purification

Laemmli sample buffer	30 ml	Bio-Rad, cat. Nr. 1610737
2-Mercaptoethanol	25 ml	Bio-Rad, cat. Nr. 1610710
SDS	100 g	Bio-Rad, cat. Nr. 1610301
Calibration syringes	200	Bio-Rad, cat. Nr. 3551174

Gel electrophoresis

Criterion™ XT 12 % Bis-Tris 1 gel - 18 wells		Bio-Rad, cat. Nr. 3450118
XT-MOPS (running buffer) (20 x)	500 ml	Bio-Rad, cat. Nr. 1610788
Kaleidoscope™ prestained standard	500 µl	Bio-Rad, cat. Nr. 1610375
MagicMark™ XP Western Standard (Molecular weight standard)	250 µl	Invitrogen, cat. Nr. LC5602

Immunobloting

Ethanol (Normapur)	1L	VWR, cat. Nr. 20821-296
Tris/CAPS (transfer buffer) (10x)	1L	Bio-Rad, cat. Nr. 1610778
Filter paper (transfer paper for Criterion™ XT precast gels)	50 sheets	Bio-Rad, cat. Nr. 1704085
PVDF membrane (0.2 µm)	10 sheets	Bio-Rad, cat. Nr. 1620175
Tween® 20	100 ml	Bio-Rad, cat. Nr. 1706531
PBS (washing buffer) (10x)	1 L	Bio-Rad, cat. Nr. 1610780
ECL (substrate for conjugate)	125 ml	Amersham, cat. Nr. RPN2109
ECL Hyperfilms (18 x 24 cm)	25 films	Amersham, cat. Nr. RPN2103K
Development folders	30 folders	Applied Biosystems, cat. Nr. T2258
Kodak developing solution LX24	to 20 L	VWR or Kodak
Kodak fixative solution AL4	to 20 L	VWR or Kodak

6.1.2 - EQUIPMENT

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

Sample purification

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

Gel electrophoresis

Criterion™ XT Cell	Bio-Rad, cat. Nr. 1656001
PowerPac™ HC power supply: 100/120 V - 220/240 V	Bio-Rad, cat. Nr. 1645052

Transfer

Criterion™ XT blotter	Bio-Rad, cat. Nr. 1704070

Immunoblotting

Western Processor base	Bio-Rad, Discontinued
Western Processor Criterion™ XT kit	Bio-Rad, Discontinued

6.2 - PREPARATION OF REAGENTS
6.2.1 - SAMPLE PURIFICATION

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

- ▶ 1 ml Reagent A
- ▶ 20 µl Proteinase K

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

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

- ▶ 0.6 g SDS
- ▶ 1.5 ml 2-Mercaptoethanol

Mix by inverting.

- ▶ 28.5 ml Laemmli sample buffer

Solution is aliquoted into 4 ml aliquots and stored at -20°C. Thawed aliquots can be re-frozen.
Note: It is recommended to prepare Laemmli solution one hour before use allowing SDS to be correctly dissolved.

6.2.2 - ELECTROPHORESIS

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

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

• Criterion™ XT migration buffer
Solution of MOPS (1x).
Prepare a 1/20 dilution. 1 L of diluted buffer is required for 1 tank:

- ▶ 950 ml Distilled water
- ▶ 50 ml MOPS buffer (20x)

Homogenize. Solution can not be stored.

6.2.3 - PROTEIN TRANSFER

• Transfer buffer
Solution of Tris/CAPS-Ethanol 15%. **Approximately 2 L is required for 1 migration tank**.

- ▶ 750 ml Distilled water
- ▶ 150 ml Pure ethanol
- ▶ 100 ml Tris/CAPS (10x)

Homogenize. Solution can not be stored.

6.2.4 - IMMUNOBLOTTING

• Wash solution 1
Solution of PBS (1x) + 0.1% Tween® 20. **Approximately 1 L is required for the complete process of 1 membrane**.

- ▶ 900 ml Distilled water
- ▶ 100 ml PBS (10x)
- ▶ 1 ml Tween® 20

Thorougly homogenize. Solution is stored at +2°C to +8°C, overnight.

• Wash solution 2
Solution of PBS (1x). **Approximately 200 ml is required for the complete process of 1 membrane**.

- ▶ 900 ml Distilled water
- ▶ 100 ml PBS (10x)

Solution is stored at room temperature (+18°C to +30°C) overnight.

• Blocking solution
During the transfer step, dilute the blocking solution (BI) 1/10 in Wash solution 1. **40 ml of diluted blocking solution is required for 1 membrane**.

- ▶ 36 ml Wash solution 1
- ▶ 4 ml Blocking solution (10x)

Homogenize by tube inverting.

• Diluted primary antibody

Just prior to use, dilute the primary antibody 1/10 in Wash solution 1.
30 ml of diluted antibody is required for 1 membrane.

- ▶ 27 ml Wash solution 1
- ▶ 3 ml Primary antibody (10x)

Homogenize by inverting.

• Diluted secondary antibody (conjugate)

Just prior to use, dilute the secondary antibody 1/10 in Wash solution 1.
40 ml of diluted conjugate is required for 1 membrane.

- ▶ 36 ml Wash solution 1
- ▶ 4 ml Secondary antibody (10x)

Homogenize by tube inverting.

• ECL

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

- ▶ 1 ml Reagent 1
- ▶ 1 ml Reagent 2

Homogenize.

• Development solution

- ▶ 800 ml Distilled water
- ▶ 200 ml Development product

Solution is stored at room temperature (+18°C to +30°C), in a darkroom for 15 days maximum.

• Fixative solution

- ▶ 800 ml Distilled water
- ▶ 200 ml Fixative product

Solution is stored at room temperature (+18°C to +30°C), in a darkroom for 15 days maximum.

6.3 - SAMPLE PURIFICATION

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

Sampling

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

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

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

Sample grinding

Place the tubes in the crown of the homogenizer.

Perform one agitation cycle with the following instrument parameters.

	Ribolyser®		**TeSeE™ PRECESS 48™ or TeSeE™ PRECESS 24™**	
	Nervous tissues	Peripheral tissues	Nervous tissues	Peripheral tissues
Time (sec.)	45	2 x 45[1]	-	-
Speed	6.5	6.5	-	-
Program	-	-	Program 1	Program 2

When grinding is insufficient, another 1 or 2 agitation cycles can be performed[2].

[1] [2] Wait a 5 minutes pause between the 2 agitation cycles.

Sample calibration

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

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

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

- At room temperature (+18°C to +30°C) for 15 hours.

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

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

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

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

Concentration of the PrP^{res} by centrifugation
Centrifuge the tubes for 7 minutes at 15000 g at 20°C.

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

6.4 - ELECTROPHORESIS

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

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

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

6.5 - PROTEIN TRANSFER

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

Protein transfer preparation
Cut the membrane to the gel dimensions. Always use forceps when handling the membrane.
Immerse the membrane in pure ethanol for 15 seconds, rinse in distilled water for 5 minutes, then for 10 minutes in the transfer buffer.
Carefully remove the gel from the plastic plates and let it equilibrate for 10 minutes in the transfer buffer.

Gel sandwich preparation
Soak filter paper and fibre pads in the transfer buffer.
Open the transfer cassette, with red side on the left. Respectively place on the red side a fiber pad, a filter paper, the membrane* and the gel*.
Complete with a filter paper then a fibre pad and close the cassette.
Immerse it in the transfer tank, previously filled to the indicated limit with transfer buffer. A frozen ice pack is added prior to fill the tank.
*Remove any air bubbles which may have formed.
Note: In case several membranes are processed at the same time, label each membrane in the corner.

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

6.6 - IMMUNOBLOTTING

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

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

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

d) Eliminate the primary antibody solution and using Wash solution 1, briefly rinse the membrane, then wash twice for respectively 5 and 10 minutes, under fast agitation.
100 ml of Wash solution 1 is required for each cycle and for 1 membrane.

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

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

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

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

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

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

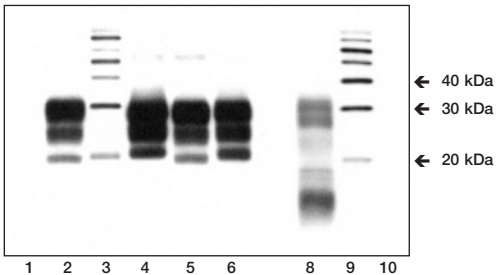
k) Immerse the film in developing solution for 45 seconds (see paragraph 6.2.4). Rinse in distilled water. Immerse the film in fixative solution until the film becomes completely transparent.

l) Wash with distilled water and let the film dry.

7 - INTERPRETATION OF RESULTS

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

Figure 1



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

Positive samples were also all treated with proteinase K.

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

Ovine sample experimentally infected with BSE (position 5) is presenting a higher signal on the di-glycosylated than on the mono-glycosylated band. Nevertheless, this typical glycoprofile can not be considered as a sufficient proof the infection of the animal with BSE. According to the Community Reference Laboratory (CRL) recommendations, a differentiation assay must be performed on this type of sample to conclude between scrapie and BSE. Please contact Bio-Rad for more information on the Bio-Rad Discriminatory test.

Atypical scrapie (e.g. Nor98) affected ovine sample (position 8) is presenting an atypical glycoprofile. A lower band is visible at approximately 12 kDa, while other upper bands are not located at the same positions compare to "typical" scrapie cases. Signal is also stronger on the lowest band than on the upper band.
Gel reading must be considered cautiously since a strong positive sample detected with the TeSeE™ Western Blot assay may hide the nearest negative or low positive sample.

Limits of the test:
A negative result means that the test sample does not contain detectable PrP^{res} by TeSeE™ Western Blot assay. However, as very low levels of PrP^{res} may not be detected, such a result does not exclude the possibility of infection.
Any sample with a negative result according to the TeSeE™ Western Blot assay interpretation criteria, following a positive rapid test result, should be tested with one of the other OIE certified confirmatory methods, Immunohistochemistry (IHC) or SAF-Immunoblot.
Any sample with a reproducible positive result according to the test interpretation critera must be verified in accordance with current legal regulation.

8 - PRECAUTIONS

The quality of the data obtained depends on compliance with the following good laboratory practices:
- Reagents must be stored at the appropiate temperature (refer to supplier's indications).
- Do not use reagents whose shelf-life has expired.
- Do not use reconstituted proteinase K after 10 hours storage at room temperature (+18°C to +30°C).
- Do not mix or combine reagents derived from different batches of the TeSeE™ Western Blot assay during the same manipulation, with the exception of grinding tubes, reagent A, reagent B and proteinase K.
- Allow the reagents and buffers to adjust to room temperature (+18°C to +30°C) for 30 minutes before use.
- Thoroughly reconstitute reagents, avoiding any contamination.
- Do not perform the test in the presence of reactive vapors (acids, alkalines, aldehydes) or dust, which could alter the enzymatic activity of the conjugate.

- The enzymatic reaction is very sensitive to all metals or metallic ions. Consequently, no metallic element must be in contact with the conjugate.
- Only use polypropylene tubes.
- Use clean glassware, rinsed in distilled water, or preferably disposable material.
- Use a new pipette tip for each sample.
- When starting electrophoresis and transfer, check that the 2 electrodes are in contact with buffer.
- All the rinsing times must be respected to avoid any excess background noise during final staining with ECL reagent.

9 - HYGIENE AND SAFETY INSTRUCTIONS

Generally, hygiene conditions, biosafety measures and good laboratory practices must be in agreement with the recommendations of national regulatory authorities.

- All reagents of the kit are intended for use in "*in vitro*" diagnosis.
- Wear disposable gloves when handling reagents and samples and wash your hands thoroughly after handling them.
- Do not pipette by mouth.
- Use polypropylene containers to avoid broken glass.
- All the materials directly in contact with the samples and the wash solutions must be considered as contaminated.
- Avoid splashing samples or solutions containing samples.
- Contaminated surfaces must be cleaned with 20 000 ppm sodium hypochlorite solution (bleach). When the contaminating liquid is an acid, contaminated surfaces must be first neutralized with sodium hydroxide before using bleach. Surfaces must be rinsed with distilled water, dried with ethanol and wiped with absorbent paper. The material used for cleaning must be discarded in a specific container for contaminated waste.
- Samples, material and contaminated products must be eliminated after decontamination:
  - either by soaking in 1 M sodium hydroxide (final concentration) for at least 1 hour at room temperature (+18°C to +30°C),
  - or by soaking in 20 000 ppm sodium hypochlorite solution for at least 1 hour at room temperature (+18°C to +30°C),
  - or by autoclaving at 134°C minimum for at least 18 minutes, under 3 bars of pressure.

  Note: never autoclave solutions containing bleach or reagent B.

- All operations involved in Transmissible Spongiform Encephalopathy (TSE) screening tests are subject to regulations and must be performed in an isolated, limited and controlled access laboratory devoted exclusively to this activity. A laboratory coat, overshoes, gloves, mask with visor or simple mask with safety glasses are required to ensure the operator's safety.
- Operators must receive specific training concerning the risks related to TSEs agents or prions and the validated modes of decontamination for unconventional agents. Biosafety measures must be in agreement with recommendations of regular authorities of the country.
- Neutralize and/or autoclave all wash solutions or wash wastes or any liquid containing biological samples prior to their elimination.
- For hazard and precaution recommendations relating to this test kit, please refer to the pictogram(s) displayed on reagent labels and the information supplied at the end of this instructions for use document. The Safety Data Sheet is available on www.bio-rad.com.

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(CN) Traditional • 本產品包含人/動物成分，請小心處理。

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(EE) • Käesolev toode sisaldab inim-või loomseid komponente. Käsitseda ettevaatlikult.

(EN) • This product contains human or animal components. Handle with care.

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(HU) • A készítmény emberi vagy állati eredetű összetevőket tartalmaz. Óvatosan kezelendő.

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H226 - H318 - H315 - H334 - H412
P210 - P261 - P280
P305+P351+P338
P302+P352 - P273 - P501

(BG)
опасно
Запалими течност и пари. Предизвиква сериозно увреждане на очите. Предизвиква дразнене на кожата. Може да причини алергични или астматични симптоми или затруднения в дишането при вдишване Вреден за водните организми, с дълготраен ефект.
Да се пази от топлина. Тютюнопушенето забранено. Избягвайте вдишване на прах/пушек/газ/дим/изпарения/аерозоли Използвайте предпазни ръкавици/предпазно облекло/предпазни очила/предпазна маска за лице. ПРИ КОНТАКТ С ОЧИТЕ: Промивайте внимателно с вода в продължение на няколко минути. Свалете контактните лещи, ако има такива и доколкото това е възможно. Продължавайте да промивате. ПРИ КОНТАКТ С КОЖАТА: Измийте обилно със сапун и вода. Да се избягва изпускане в околната среда. Изхвърлете съдържанието/контейнера в съответствие с местните/регионалните/националните/международните разпоредби.

(CN)
危险
易燃液体和蒸气. 引起严重的眼睛损伤.
引起皮肤刺激
吸入可能引起过敏或哮喘症状或呼吸困难. 对水生生物有害并且有长期持续影响. 远离热源/火花/明火/热表面。 – 禁止吸烟。. 避免吸入粉尘/烟/气体/烟雾/蒸气/喷雾。. 戴防护手套/穿防护服/戴防护眼罩/戴防护面具。. 如进入眼睛：用水小心冲洗几分钟。如戴隐型眼镜并可方便地取出，取出隐型眼镜。继续冲洗。 如皮肤沾染：用大量肥皂和水清洗。. 避免释放到环境中。. 按照本地 / 地区 / 国家 / 国际规例处理内含物 / 容器。

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危險
易燃液體和蒸氣. 引起嚴重的眼睛損傷.
引起皮膚刺激. 吸入可能引起過敏或哮喘症狀或呼吸困難. 對水生生物有害並且有長期持續影響. 遠離熱源/火花/明火/熱表面。 – 禁止吸煙。. 避免吸入粉塵/煙/氣體/煙霧/蒸氣/噴霧。. 戴防護手套/穿防護服/戴防護眼罩/戴防護面具。. 如進入眼睛：用水小心沖洗幾分鐘。如戴隱型眼鏡並可方便地取出，取出隱型眼鏡。繼續沖洗。 如皮膚沾染：用大量肥皂和水清洗。. 避免釋放到環境中。. 按照本地 / 地區 / 國家 / 國際規例處理內含物 / 容器。

(CZ)
Nebezpečí
Hořlavá kapalina a páry. Způsobuje vážné poškození očí. Dráždí kůži. Při vdechování může vyvolat příznaky alergie nebo astmatu nebo dýchací potíže. Škodlivý pro vodní organismy, s dlouhodobými účinky.
Chraňte před teplem/jiskrami/otevřeným plamenem/horkými povrchy. Zákaz kouření. Zamezte vdechování prachu/dýmu/plynu/mlhy/par/aerosolů. Používejte ochranné rukavice/ochranný oděv/ochranné brýle/obličejový štít. PŘI ZASAŽENÍ OČÍ: Několik minut opatrně vyplachujte vodou. Vyjměte kontaktní čočky, jsou-li nasazeny a pokud je lze vyjmout snadno. Pokračujte ve vyplachování. PŘI STYKU S KŮŽÍ: Omyjte velkým množstvím vody a mýdla. Zabraňte uvolnění do životního prostředí. Obsah/nádobu likvidujte v souladu s místními/regionálními/národními/mezinárodními předpisy.

(DE)
Gefahr
Flüssigkeit und Dampf entzündbar. Verursacht schwere Augenschäden. Verursacht Hautreizungen. Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen. Schädlich für Wasserorganismen, mit langfristiger Wirkung.
Von Hitze/Funken/offener Flamme/heißen Oberflächen fernhalten. Nicht rauchen. Einatmen von Staub/Rauch/Gas/Nebel/Dampf/Aerosol vermeiden. Schutzhandschuhe/Schutzkleidung/Augenschutz/Gesichtsschutz tragen. BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen. BEI KONTAKT MIT DER HAUT: Mit viel Wasser und Seife waschen. Freisetzung in die Umwelt vermeiden. Entsorgung des Inhalts / des Behälters gemäß den örtlichen / regionalen / nationalen/ internationalen Vorschriften.

(DK)
Fare
Brandfarlig væske og damp. Forårsager alvorlig øjenskade. Forårsager hudirritation. Kan forårsage allergi- eller astmasymptomer eller åndedrætsbesvær ved indånding. Skadelig for vandlevende organismer, med langvarige virkninger. Holdes væk fra varme/gnister/åben ild/varme overflader. Rygning forbudt. Undgå indånding af pulver/røg/gas/tåge/damp/spray. Bær beskyttelseshandsker/beskyttelsestøj/øjenbeskyttelse/ansigtsbeskyttelse VED KONTAKT MED ØJNENE: Skyl forsigtigt med vand i flere minutter. Fjern eventuelle kontaktlinser, hvis dette kan gøres let. Fortsæt skylning. VED KONTAKT MED HUDEN: Vask med rigeligt sæbe og vand. Undgå udledning til miljøet. Bortskaffelse af indholdet/beholderen i henhold til de lokale/regionale/nationale/internationale forskrifter.

(EE)
Ettevaatust
Tuleohtlik vedelik ja aur. Põhjustab raskeid silmakahjustusi. Põhjustab nahaärritust. Sissehingamisel võib põhjustada allergia- või astma sümptomeid või hingamisraskusi. Ohtlik veeorganismidele, pikaajaline toime.
Hoida eemal soojusallikast/sädemetest/leekidest/kuumadest pindadest. Mitte suitsetada. Vältida tolmu/suitsu/gaasi/udu/auru/pihustatud aine sissehingamist. Kanda kaitsekindaid/kaitserõivastust/kaitseprille/kaitsemaski. SILMA SATTUMISE KORRAL: loputada mitme minuti jooksul ettevaatlikult veega. Eemaldada kontaktläätsed, kui neid kasutatakse ja kui neid on kerge eemaldada. Loputada veel kord. NAHALE SATTUMISE KORRAL: pesta rohke vee ja seebiga. Vältida sattumist keskkonda. Sisu/konteineri käitlus vastavuses kohalike/regionaalsete/rahvuslike/rahvusvaheliste nõuetega.

(EN)
Danger
Flammable liquid and vapour. Causes serious eye damage. Causes skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Harmful to aquatic life with long lasting effects.
Keep away from heat/sparks/open flames/hot surfaces. No smoking. Avoid breathing dust/fume/gas/mist/vapours/spray. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN: Wash with plenty of soap and water. Avoid release to the environment. Dispose of contents/container in accordance with local/regional/national/international regulations.

(ES)
Peligro
Líquidos y vapores inflamables. Provoca lesiones oculares graves. Provoca irritación cutánea. Puede provocar síntomas de alergia o asma o dificultades respiratorias en caso de inhalación. Nocivo para los organismos acuáticos, con efectos nocivos duraderos.
Mantener alejado de fuentes de calor/chispas/llama abierta/superficies calientes. No fumar. Evitar respirar el polvo/el humo/el gas/la niebla/los vapores/el aerosol. Llevar guantes que aíslen del frío/gafas/máscara. EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando. EN CASO DE CONTACTO CON LA PIEL: Lavar con agua y jabón abundantes. Evitar su liberación al medio ambiente. Eliminar el contenido o el recipiente conforme a la reglamentación local/regional/nacional/internacional.

(FI)
Vaara
Syttyvä neste ja höyry. Vaurioittaa vakavasti silmiä. Ärsyttää ihoa. Voi aiheuttaa hengitettynä allergia- tai astmaoireita tai hengitysvaikeuksia. Haitallista vesieliöille, pitkäaikaisia haittavaikutuksia. Suojaa lämmöltä/kipinöiltä/avotulelta/kuumilta pinnoilta. Tupakointi kielletty. Vältä pölyn/savun/kaasun/sumun/höyryn/suihkeen hengittämistä. Käytä suojakäsineitä/suojavaatetusta/silmiensuojainta/kasvonsuojainta. JOS KEMIKAALIA JOUTUU SILMIIN: Huuhdo huolellisesti vedellä usean minuutin ajan. Poista piilolinssit, _edical voi tehdä helposti. Jatka huuhtomista. JOS KEMIKAALIA JOUTUU IHOLLE: Pese runsaalla vedellä ja saippualla. Vältettävä päästämistä ympäristöön. Säilytä säiliö(t) noudattaen paikallisia/alueellisia/kansallisia/kansainvälisiä määräyksiä.

(FR)
Danger
Liquide et vapeurs inflammables. Provoque des lésions oculaires graves. Provoque une irritation cutanée. Peut provoquer des symptômes allergiques ou d'asthme ou des difficultés respiratoires par inhalation. Nocif pour les organismes aquatiques, entraîne des effets néfastes à long terme. Tenir à l'écart de la chaleur/des étincelles/des flammes nues/des surfaces chaudes. Ne pas fumer. Éviter de respirer les poussières/fumées/gaz/brouillards/vapeurs/aérosols. Porter des gants de protection/des vêtements de protection/un équipement de protection des yeux/du visage. EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer. EN CAS DE CONTACT AVEC LA PEAU: laver abondamment à l'eau et au savon. Éviter le rejet dans l'environnement. Éliminer le contenu/récipient conformément à la réglementation locale/régionale/nationale/internationale.

(GR)
Κίνδυνος
Υγρό και ατμοί εύφλεκτα. Προκαλεί σοβαρή οφθαλμική βλάβη. Προκαλεί ερεθισμό του δέρματος. Μπορεί να προκαλέσει αλλεργία ή συμπτώματα άσθματος ή δύσπνοια σε περίπτωση εισπνοής. Επιβλαβές για τους υδρόβιους οργανισμούς, με μακροχρόνιες επιπτώσεις. Μακριά από θερμότητα/σπινθήρες/γυμνές φλόγες/θερμές επιφάνειες. Μην καπνίζετε. Αποφεύγετε να αναπνέετε σκόνη/αναθυμιάσεις/αέρια/σταγονίδια/ατμούς/εκνεφώματα. Να φοράτε προστατευτικά γάντια/προστατευτικά ενδύματα/μέσα ατομικής προστασίας για ταμάτια/πρόσωπο. ΣΕ ΠΕΡΙΠΤΩΣΗ ΕΠΑΦΗΣ ΜΕ ΤΑ ΜΑΤΙΑ: Ξεπλύνετε προσεκτικά με νερό για αρκετά λεπτά. Εάν υπάρχουν φακοί επαφής, αφαιρέστε τους, εφόσον είναι εύκολο. Συνεχίστε να ξεπλένετε. ΣΕ ΠΕΡΙΠΤΩΣΗ ΕΠΑΦΗΣ ΜΕ ΤΟ ΔΕΡΜΑ: Πλύνετε με άφθονο νερό και σαπούνι. Να αποφεύγεται η ελευθέρωση στο περιβάλλον. Απορρίψτε τα περιεχόμενα/δοχείο σύμφωνα με τους τοπικούς/εθνικούς/διεθνείς κανονισμούς.

(HR)
Opasnost
Zapaljiva tekućina i para. Uzrokuje teške ozljede oka. Nadražuje kožu. Ako se udiše može izazvati simptome alergije ili astme ili poteškoće s disanjem Štetno za vodeni okoliš s dugotrajnim učincima.
Čuvati odvojeno od topline/iskre/otvorenog plamena/vrućih površina. – Ne pušiti. Izbjegavati udisanje prašine/dima/plina/magle/pare/aerosola. Nositi zaštitne rukavice/zaštitnu odijelo/zaštitu za oči/zaštitu za lice. U SLUČAJU DODIRA S OČIMA: oprezno ispirati vodom nekoliko minuta. Ukloniti kontaktne leće ukoliko ih nosite i ako se one lako uklanjaju. Nastaviti isprinje. U SLUČAJU DODIRA S KOŽOM: oprati velikom količinom sapuna i vode. Izbjegavati ispuštanje u okoliš. Odložite sadržaje /spremnike u skladu s lokalnim/regionalnim/nacionalni/međunarodnim odredbama.

(HU)
Veszély
Tűzveszélyes folyadék és gőz. Súlyos szemkárosodást okoz. Bőrirritáló hatású. Belélegezve allergiás és asztmás tüneteket, és nehéz légzést okozhat. Ártalmas a vízi élővilágra, hosszan tartó károsodást okoz.
Hőtől/szikrától/nyílt lángtól/forró felületektől távol tartandó. Tilos a dohányzás. Kerülje a por/füst/gáz/köd/gőzök/permet belélegzését. Védőkesztyű/védőruha/szemvédő/arcvédő használata kötelező. SZEMBE KERÜLÉS esetén: Több percig tartó óvatos öblítés vízzel. Adott esetben a kontaktlencsék eltávolítása, ha könnyen megoldható. Az öblítés folytatása. HA BŐRRE KERÜL: Lemosás bő szappanos vízzel. Kerülni kell az anyagnak a környezetbe való kijutását. Az edény tartalmát / a tartályt a helyi/regionális/nemzeti/nemzetközi szabályozásoknak megfelelően kell hulladékként elhelyezni.

(IT)
Pericolo
Liquido e vapori infiammabili. Provoca gravi lesioni oculari. Provoca irritazione cutanea. Può provocare sintomi allergici o asmatici o difficoltà respiratorie se inalato. Nocivo per gli organismi acquatici con effetti di lunga durata. Tenere lontano da fonti di calore/scintille/fiamme libere/superfici riscaldate. Non fumare. Evitare di respirare la polvere/i fumi/i gas/la nebbia/i vapori/gli aerosol. Indossare guanti/indumenti protettivi/Proteggere gli occhi/il viso. IN CASO DI CONTATTO CON GLI OCCHI: sciacquare accuratamente per parecchi minuti. Togliere le eventuali lenti a contatto se è agevole farlo. Continuare a sciacquare. IN CASO DI CONTATTO CON LA PELLE: lavare abbondantemente con acqua e sapone. Non disperdere nell'ambiente. Smaltire il prodotto/recipiente in conformità con le disposizioni locali / regionali / nazionali / internazionali.

(JP)
危険
引火性液体及び蒸気. 重篤な眼の損傷.
皮膚刺激. 吸入するとアレルギー，ぜん（喘）息又は呼吸困難を起こすおそれ. 長期継続的影響によって水生生物に有害. 熱 / 火花 / 裸火 / 高温のもののような着火源から遠ざけること。-禁煙。. 粉じん/煙/ガス/ミスト/蒸気/スプレーの吸入を避けること。. 保護手袋/保護衣/保護眼鏡/顔保護面の着用。. 眼に入った場合：水で数分間注意深く洗うこと。次にコンタクトレンズを着用していて容易に外せる場合は外すこと。その後も洗浄を続けること。 皮膚に付着した場合：多量の水と石けん（鹸）で洗うこと。. 環境への放出を避けること。. 現地/地域/国/国際規定に従い内容物·容器の露出.

(KR)
위험
인화성 액체 및 증기. 눈에 심한 손상을 일으킴.
피부에 자극을 일으킴. 흡입시 알레르기성 반응, 천식 또는 호흡 곤란을 일으킬 수 있음. 장기적인 영향에 의해 수생생물에게 유해함. 열·스파크·화염·고열로부터 멀리하시오 – 금연. (분진·흄·가스·미스트·증기·스프레이)의 흡입을 피하시오. (보호장갑·보호의·보안경·안면보호구)를(을) 착용하시오. 눈에 묻으면 몇 분간 물로 조심해서 씻으시오. 가능하면콘택트렌즈를 제거하시오. 계속 씻으시오. 피부에 묻으면 다량의 비누와 물로 씻으시오. 환경으로 배출하지 마시오. 현지/지역/국가/국제규정에 따라서 내용물/용기 노출.

(LT)
Pavojinga
Degūs skystis ir garai. Smarkiai pažeidžia akis. Dirgina odą. Įkvėpus gali sukelti alerginę reakciją, astmos simptomus arba apsunkinti kvėpavimą. Kenksminga vandens organizmams, sukelia ilgalaikius pakitimus.
Laikyti atokiau nuo šilumos šaltinių/žiežirbų/atviros liepsnos/karštų paviršių. Nerūkyti. Stengtis neįkvėpti dulkių/dūmų/dujų/rūko/garų/aerozolio. Mūvėti apsaugines pirštines/dėvėti apsauginius drabužius/naudoti akių (veido) apsaugos priemones. PATEKUS Į AKIS: Kelias minutes atsargiai plauti vandeniu. Išimti kontaktinius lęšius, jeigu jie yra ir jeigu lengvai galima tai padaryti. Toliau plauti akis. PATEKUS ANT ODOS: Nuplauti dideliu kiekiu muilo ir vandens. Saugoti, kad nepatektų į aplinką. Turinį/talpą išpilti (išmesti) - šalinti pagal vietines / regionines / nacionalines / tarptautines taisykles.

(NL)
Gevaar
Ontvlambare vloeistof en damp. Veroorzaakt ernstig oogletsel. Veroorzaakt huidirritatie. Kan bij inademing allergie- of astmasymptomen of ademhalingsmoeilijkheden veroorzaken. Schadelijk voor in het water levende organismen, met langdurige gevolgen.
Verwijderd houden van warmte/vonken/open vuur/hete oppervlakken. Niet roken. Inademing van stof/rook/gas/nevel/damp/spuitnevel vermijden. Beschermende handschoenen/beschermende kleding/oogbescherming/gelaatsbescherming

dragen. BIJ CONTACT MET DE OGEN: voorzichtig afspoelen met water gedurende een aantal minuten; contactlenzen verwijderen, indien mogelijk; blijven spoelen. BIJ CONTACT MET DE HUID: met veel water en zeep wassen. Voorkom lozing in het milieu. De inhoud en de verpakking verwerken volgens de plaatselijke/regionale/nationale/internationale voorschriften.

(NO)
Fare
Brennbar væske og damp. Forårsaker alvorlige øyeskader. Irriterer huden. Kan forårsake allergi, astmalignende symptomer eller pusteproblemer ved innånding. Skadelig for vannlevende organismer, langtidsvirkning. Holdes adskilt fra varme. Ikke røyk. Unngå innånding av støv/røyk/gass/sprøytetåke/damp/aerosol. Bruk vernehansker/verneklær/vernebriller/ansiktsskjerm. VED KONTAKT MED ØYNENE: Skyll forsiktig med vann i opptil flere minutter. Fjern evt. kontaktlinser såfremt dette er lett mulig. Fortsett skyllingen. VED HUDKONTAKT: Vask med store mengder vann og såpe. Unngå utslipp til miljøet. Innholdet / emballasjen skal avhendes i henhold til de lokale / regionale / nasjonale / internasjonale forskrifter.

(PL)
Niebezpieczeństwo
Łatwopalna ciecz i pary. Powoduje poważne uszkodzenie oczu. Działa drażniąco na skórę. Może powodować objawy alergii lub astmy lub trudności w oddychaniu w następstwie wdychania. Działa szkodliwie na organizmy wodne, powodując długotrwałe skutki. Przechowywać z dala od źródeł ciepła/iskrzenia/otwartego ognia/gorących powierzchni. Palenie wzbronione. Unikać wdychania pyłu/dymu/gazu/mgły/par/rozpylonej cieczy. Stosować rękawice ochronne/odzież ochronną/ochronę oczu/ochronę twarzy. W PRZYPADKU DOSTANIA SIĘ DO OCZU: Ostrożnie płukać wodą przez kilka minut. Wyjąć soczewki kontaktowe, jeżeli są i można je łatwo usunąć. Nadal płukać. W PRZYPADKU KONTAKTU ZE SKÓRĄ: Umyć dużą ilością wody z mydłem. Unikać uwolnienia do środowiska. Zawartość / pojemnik usuwać zgodnie z przepisami miejscowymi / regionalnymi / narodowymi / międzynarodowymi.

(PT)
Perigo
Líquido e vapor inflamáveis. Provoca lesões oculares graves. Provoca irritação cutânea. Quando inalado, pode provocar sintomas de alergia ou de asma ou dificuldades respiratórias. Nocivo para os organismos aquáticos com efeitos duradouros. Manter afastado do calor/da faísca/da chama aberta/das superfícies quentes. Não fumar. Evitar respirar as poeiras/fumos/gases/névoas/vapores/aerossóis. Usar luvas de protecção/vestuário de protecção/protecção ocular/protecção facial. SE ENTRAR EM CONTACTO COM OS OLHOS: enxaguar cuidadosamente com água durante vários minutos. Se usar lentes de contacto, retire-as, se tal lhe for possível. Continuar a enxaguar. SE ENTRAR EM CONTACTO COM A PELE: lavar com sabonete e água abundantes. Evitar a libertação para o ambiente. Eliminar o conteúdo/recipiente de acordo com a legislação local/regional/nacional/internacional.

(RO)
Pericol
Lichid şi vapori inflamabili. Provoacă leziuni oculare grave. Provoacă iritarea pielii. Poate provoca simptome de alergie sau astm sau dificultăţi de respiraţie în caz de inhalare. Nociv pentru mediul acvatic cu efecte pe termen lung. A se păstra departe de surse de căldură/scântei/flăcări deschise/suprafeţe încinse. Fumatul interzis. Evitaţi să inspira i praful/fumul/gazul/cea a/vaporii/spray-ul. Purtaţi mănuşi de protecţie/îmbrăcăminte de protecţie/echipament de protecţie a ochilor/ chipament de protecţie a feţei. ÎN CAZ DE CONTACT CU OCHII: clătiţi cu atenţie cu apă timp de mai multe minute. Scoateţi lentilele de contact, dacă este cazul şi dacă acest lucru se poate face cu uşurinţă. Continuaţi să clătiţi. ÎN CAZ DE CONTACT CU PIELEA: spălaţi cu multă apă şi săpun. Evitaţi dispersarea în mediu. Aruncaţi conţinutul/containerul în acord cu regulamentele locale/regionale/naţionale/internaţionale.

(SE)
Fara
Brandfarlig vätska och ånga. Orsakar allvarliga ögonskador. Irriterar huden. Kan orsaka allergi- eller astmasymtom eller andningssvårigheter vid inandning. Skadliga långtidseffekter för vattenlevande organismer. Får inte utsättas för värme/gnistor/öppen låga/heta ytor. Rökning förbjuden. Undvik att inandas damm/rök/gaser/dimma/ångor/sprej. Använd skyddshandskar/skyddskläder/ögonskydd/ansiktsskydd. VID KONTAKT MED ÖGONEN: Skölj försiktigt med vatten i flera minuter. Ta ur eventuella kontaktlinser om det går lätt. Fortsätt att skölja. VID HUDKONTAKT: Tvätta med mycket tvål och vatten. Undvik utsläpp till miljön. Innehållet / behållaren avfallshanteras enligt lokala / regionala / nationella / internationella föreskrifter.

(SI)
Nevarno
Vnetljiva tekočina in hlapi. Povzroča hude poškodbe oči. Povzroča draženje kože. Lahko povzroči simptome alergije ali astme ali težave z dihanjem pri vdihavanju. Škodljivo za vodne organizme, z dolgotrajnimi učinki.
Hraniti ločeno od vročine/isker/odprtega ognja/vročih površin. Kajenje prepovedano. Ne vdihavati prahu/dima/plina/meglice/hlapov/razpršila. Nositi zaščitne rokavice/zaščitno obleko/zaščito za oči/zaščito za obraz. PRI STIKU Z OČMI: previdno izpirajte z vodo nekaj minut. Odstranite kontaktne leče, če jih imate in če to lahko storite brez težav. Nadaljujte z izpiranjem. PRI STIKU S KOŽO: umiti z veliko mila in vode. Preprečiti sproščanje v okolje. Vsebino/vsebnik odstranite v skladu z lokalnimi/regionalnimi/narodnimi/mednarodnimi predpisi.

(SK)
Nebezpečenstvo
Horľavá kvapalina a pary. Spôsobuje vážne poškodenie očí. Dráždi kožu. Pri vdýchnutí môže vyvolať alergiu alebo príznaky astmy, alebo dýchacie ťažkosti. Škodlivý pre vodné organizmy, s dlhodobými účinkami. Uchovávajte mimo dosahu tepla/iskier/otvoreného ohňa/horúcich povrchov. Nefajčite. Zabráňte vdychovaniu prachu/dymu/plynu/hmly/pár/aerosólov. Noste ochranné rukavice/ochranný odev/ochranné okuliare/ochranu tváre. PO ZASIAHNUTÍ OČÍ: Niekoľko minút ich opatrne vyplachujte vodou. Ak používate kontaktné šošovky a ak je to možné, odstráňte ich. Pokračujte vo vyplachovaní. PRI KONTAKTE S POKOŽKOU: Umyte veľkým množstvom vody a mydla. Zabráňte uvoľneniu do životného prostredia. Zneškodnenie obsahu/obalu v súlade s miestnymi/oblastnými/národnými/medzinárodnými nariadeniami.

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