TeSeE™ SAP Combi Kit
Short Assay Protocol

Σ 192	REF 3551186
Σ 384	REF 3551192
Σ 768	REF 3551191

REAGENT KITS FOR *IN VITRO* PURIFICATION AND DETECTION OF PrP^{Sc}

Within the European Union, this test is approved as rapid test for the BSE and scrapie testing programmes on cattle, sheep and goats which are set up in accordance with Annex III, chapter A to Regulation (EC) No 999/2001.

2015/06

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1 - GENERAL INFORMATION

Transmissible Spongiform Encephalopathies (TSE's) are slow degenerative diseases of the central nervous system induced by unconventional transmissible agents (UTAs) routinely called prions.

TSEs are generally classified according to their etiology, as iatrogenic, familial and/or sporadic. Sheep scrapie has been reported in the 18th century and transmission demonstrated (including to goats). However, the modes of contamination within flocks remain obscure. TSEs were also described in deer and elk (chronic wasting disease, CWD) and in cow (Bovine Spongiform Encephalopathy, BSE).

Humans are also susceptible to certain forms of TSE. There is compelling evidence supporting that Bovine Spongiform Encephalopathy (BSE) has passed from cattle to human, probably through consumption of contaminated meat.

In addition to this variant form of Creutzfeldt-Jakob disease (vCJD), other forms in humans include the Kuru and the iatrogenic Creutzfeldt-Jakob disease.

Pure hereditary forms (such as the Gerstmann-Sträussler-Scheinker syndrome [GSS]) and/or sporadic CJD have been demonstrated in humans, but their incidences are low. We do not know if similar sporadic TSE cases exist in animals.

The main characteristics of these diseases are:

- a slowly progressive but always fatal course,
- absence of conventional infectious agents,
- progressive accumulation in the central nervous system of an abnormal isoform of the natural prion protein (PrP) called PrP^{Sc}. This isoform is characterized by particular biochemical properties and especially by an increased resistance to proteases.

The strikingly long incubation period that precedes the neurological symptoms suggests that important events of TSE pathogeneses might take place in extra nervous sites and especially in peripheral lymphoid tissues.

In spite of many unknown and/or incertitudes, the detection of abnormal PrP^{Sc} is now established as the method, to confirm TSE diagnosis. This detection is mainly achieved from post mortal collected nervous tissues.

Abnormal PrP^{Sc} has also been detected in a number of lymphoid tissue and organs: in the germinal centres of spleen, lymph nodes, tonsils, and/or mucosa-associated lymphoid tissue (but at the research area), in animal models or in scrapie sheeps, CWD deers and elks and vCJD patients.

Reagent designed by the "Commissariat à l'Énergie Atomique - CEA" (French Atomic Energy Commission), developed, produced and marketed by Bio-Rad, allow PrP^{Sc} detection on samples of nervous tissues taken from animals.

Test performed with the following reagents and accessories:

- TeSeE™ SAP Combi Kit (192 tests)	Ref.: 3551186
- TeSeE™ SAP Combi Kit (384 tests)	Ref.: 3551192
- TeSeE™ SAP Combi Kit (768 tests)	Ref.: 3551191
- Grinding Tubes (384 tubes)	Ref.: 3551139
- Grinding Tubes (768 tubes)	Ref.: 3551137
- Calibration syringe and needle (x 200)	Ref.: 3551174
or Filter plates (x 50)	Ref.: 3551179
- Deepwell microplates (x 50)	Ref.: 3590132
- Medium beads (x 2000)	Ref.: 3551171*

** Only for peripheral tissues.*

2 -TeSeE™ SAP Combi Kit

2-1 Principle

The reagents of the TeSeE™ SAP Combi Kit allow purification, concentration, solubilisation and detection of PrP^{Sc} from samples of tissues obtained from infected animals.
The TeSeE™ SAP Assay is an immuno-enzymatic technique (sandwich format) using 2 monoclonal antibodies for the detection of the abnormal prion protein, resistant to proteinase K, in tissues collected from infected animals.
The solid phase is composed of 12 strips of 8 polystyrene wells coated with the first monoclonal antibody. The second monoclonal antibody is bound to peroxidase.

2-2 Samples

- **Bovine:** purification of PrP^{Sc} is performed on samples from Central Nervous System (CNS). The BSE extraction tool (Ref.: 3551130) can be used to collect brainstem.
  Since distribution of PrP^{Sc} 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 for detailed instructions on good sampling procedure.

- **Small ruminants and cervids:** purification of PrP^{Sc} is performed on samples from Central Nervous System (CNS) or peripheral tissues (lymphoïd nodes, spleen,…). The small ruminant extraction tool (Ref.: 3551184) can be used to collect both brainstem and cerebellum.
  Since distribution of PrP^{Sc} is heterogeneous in central nervous system, obex area from brainstem must be preferably sampled for optimal detection.
  Samples are cut and weighed individually.
  Note: other tissues (tonsils, ileum, eyelid…) can be used for research purposes only.
  Samples are stored at +2°C to +8°C when purification is performed within 24 hours or can be stored frozen for several months. They can only be submitted to 3 freezing/thawing cycles. If these samples must be transported, they should be packaged in accordance with current local regulations.

2-3 Composition of the TeSeE™ SAP Combi Kits

LABELLING	TYPE OF REAGENTS	PRESENTATION 3551186 (192 tests)	3551192 (384 tests)	3551191 (768 tests)	STORAGE
Reagent A	Denaturing solution	1 vial (55 ml)	1 vial (120 ml)	1 vial (240 ml)	+2°C to +8°C
Reagent B	Clarifying solution Colouring: bromophenol blue	1 vial (55 ml)	1 vial (120 ml)	2 vials (120 ml)	+2°C to +8°C
Reagent C	Resolving buffer Colouring: malachite green	1 vial (7 ml)	1 vial (14 ml)	1 vial (28 ml)	+2°C to +8°C
PK	Proteinase K Colouring: phenol red	1 vial (0.5 ml)	2 vials (0.5 ml)	4 vials (0.5 ml)	+2°C to +8°C
R1	**Microplate:** 12 strips of 8 wells coated with an anti-PrP monoclonal antibody	2 plates	4 plates	8 plates	+2°C to +8°C
R2	**Wash solution:** 10-fold concentrated Tris-NaCl buffer pH 7.4 Preservative: ProClin™ 300 (0.01%)	1 vial (250 ml)	2 vials (250 ml)	4 vials (250 ml)	+2°C to +25°C
R3	**Negative Control:** PBS buffer pH 7.2 supplemented with BSA Preservative: ProClin™ 300 (0.1%)	1 vial (4 ml)	2 vials (4 ml)	4 vials (4 ml)	+2°C to +8°C
R4	**Positive Control:** PBS buffer pH 7.4 supplemented with non infectious synthetic peptide. Lyophilized. Preservative: ProClin™ 300 (0.1%)	1 vial (q.s. 4 ml)	2 vials (q.s. 4 ml)	4 vials (q.s. 4 ml)	+2°C to +8°C
R6	**Sample diluent:** PBS buffer pH 7.2 supplemented with BSA and phenol red Preservative: ProClin™ 300 (0.1%)	1 vial (35 ml)	1 vial (70 ml)	1 vial (140 ml)	+2°C to +8°C
R7	**Conjugate:** 10-fold concentrated peroxidase-labelled anti-PrP monoclonal antibody in PBS buffer pH 7.1 solution supplemented with bovine proteins and coloured with phenol red Preservative: ProClin™ 300 (0.1%)	1 vial (2.8 ml)	2 vials (2.8 ml)	4 vials (2.8 ml)	+2°C to +8°C
R8	**Peroxidase Substrate Buffer:** Solution of citric acid and sodium acetate pH 4.0 containing 0.015% H_2O_2 and 4% dimethylsulfoxide (DMSO)	1 vial (60 ml)	1 vial (120 ml)	2 vials (120 ml)	+2°C to +8°C
R9	**Chromogen:** Tetramethylbenzidine (TMB) solution	1 vial (5 ml)	1 vial (10 ml)	1 vial (20 ml)	+2°C to +8°C
R10	**Stop solution:** 1 N sulphuric acid	1 vial (28 ml)	1 vial (56 ml)	1 vial (112 ml)	+2°C to +8°C
	Adhesive films	**8**	**12**	**16**	

The following reagents are generic components: Reagent A, reagent B, sample diluent (R6), wash solution (R2), peroxidase substrate buffer (R8), chromogen (R9) and stop solution (R10). They can be used with all batches of the TeSeE™ SAP Kits.

2-4 Preparation of Reagents

Before use, let the reagents of the TeSeE™ SAP Combi Kits adjust to room temperature (+18°C to +30°C) for 30 minutes.

1 - Ready to use reagents

Reagent A, B , C, the negative control (R3), sample dilution solution (R6) and stop solution (R10) are ready to use.

Microplates (R1):

Before opening the sealed bag with a desiccant, let the microplate adjust to room temperature (+18°C to +30°C) in its protective packaging to avoid any water condensation in the wells. Open at the solder point and immediately return the unused rows to the sachet.
Tightly close the bag after expelling any air, then store at +2°C to +8°C.

2 - Reagents to reconstitute

Proteinase K:

Reagent A is the dilution buffer for proteinase K.
The solution must be prepared in the following way (4 µl of proteinase K in 1 ml of reagent A):

NUMBER OF SAMPLES	REAGENT A	PROTEINASE K
2	1 ml	4 µl
10	3 ml	12 µl
18	5 ml	20 µl
26	7 ml	28 µl
34	9 ml	36 µl
42	11 ml	44 µl
50	13 ml	52 µl
58	15 ml	60 µl
66	17 ml	68 µl
74	19 ml	76 µl
82	21 ml	84 µl
90	23 ml	92 µl

The volumes must be pipetted exactly. The tip containing the PK has to be correctly rinsed by successive aspiration/distribution cycles in reagent A.
After reconstitution, homogenize the solution by successive inversions until you obtain a red homogeneous solution.

Wash solution (R2):

Dilute wash solution R2 to 1/10 in distilled or ultrapure water (example 100 ml of reagent R2 in 900 ml of distilled water).

Positive control (R4):

Gently tap the vial of positive control (R4) on the laboratory bench to detach any substance adherent to the rubber stopper. Open the vial and dissolve the content in 4 ml of diluent R6. Reseal the vial and let stand for approximately 1 minute, homogenizing gently and occasionally to facilitate dissolution.

Conjugate (R7):

Dilute reagent R7 to 1/10 in the freshly reconstituted wash solution (example: 0.1 ml of reagent R7 in 0.9 ml of reconstituted wash solution) bearing in mind that 1 ml of ready-for-use conjugate is sufficient for 1 row. Homogenize gently. Avoid using a vortex agitator.

Enzymatic development solution (R8 + R9):
Dilute reagent R9 to 1/11 in reagent R8 (example: 0.1 ml of reagent R9 in 1 ml of reagent R8) bearing in mind that 1.1 ml of enzymatic revelation solution is sufficient for 1 row.
Homogenize gently. Avoid using a vortex agitator.

2-5 Storage, Shelf-life

Store the TeSeE™ SAP Combi Kits at +2°C to +8°C. All reagents are stable at this temperature until the expiry date indicated on the kit (before and after opening of the vials).
After dilution, the reconstituted proteinase K solution when stored at room temperature (+18°C to +30°C) must be used within 6 hours.

The shelf-lives of the reagents after preparation are as follows:

LABELLING	REAGENT	SHELF-LIFE
R1	Microplate in tightly closed sachet	1 month at +2°C to +8°C
R2	Diluted wash solution	24 hours at room temperature (+18°C to + 30°C) 2 weeks at +2°C to +8°C
R4	Reconstituted positive control	2 hours at room temperature (+18°C to + 30°C) 4 hours at +2°C to +8°C 6 months at -20°C It is recommended to divide the reconstituted solution into 0.5 ml aliquots and to store them immediately at -20°C. Can be submitted to 3 successive freezing/thawing cycles.
R7	Reconstituted conjugate solution (with diluted wash solution)	8 hours at room temperature (+18°C to + 30°C)
R8 + R9	Development solution	6 hours at room temperature (+18°C to + 30°C) always protected from light

2-6 Assay Procedure

For the semi-automatic processing of the purification protocol, please refer to the TeSeE™ NSP operator's manual.

Procedure for manual processing:

1. Sampling:
For peripheral tissues (lymph nodes, spleen,…) 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 samples in grinding tubes, close firmly and proceed to the grinding step in the homogenizer (Ribolyser®, TeSeE™ PRECESS 24™ or TeSeE™ PRECESS 48™ systems).

2. Sample grinding:
Place the tubes in the crown of the homogenizer (Ribolyser®, TeSeE™ PRECESS 24™ or TeSeE™ PRECESS 48™ systems). Perform one agitation cycle with the following instrument parameters:

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

* Wait a 5 minutes pause between the 2 agitation cycles.

When grinding is insufficient, another 1 or 2 agitation cycles can be performed, by ensuring that the temperature of the tube returns to room temperature (+18°C to +30°C) between each cycle (using crushed ice for example).

3. Sample transfer:
Remove the grinding tubes from the homogenizer, resuspend the homogenate by inversion before opening the tubes.
Transfer the homogenate with one of the following methods:

- **Calibration syringe method**
  Take 250 µl with the calibration syringe (Ref.: 3551174) taking care to immerse the needle in the pellet of beads to avoid sampling poorly homogenized tissue fragments.
  Transfer each 250 µl sample into a 2 ml Eppendorf micro test-tube or Deepwell (Ref.: 3590132).

- **Filter plate method**
  The transfer and the filtration are done separately using a filter plate (Ref.: 3551179) and a Deepwell plate (Ref.: 3590132), with either one of the two following filtration techniques.

- Vacuum technique:
  Fit the Deepwell plate (Ref.: 3590132) (the master plate) in the bottom of the vacuum manifold, place the lead of the manifold and then the filter plate (Ref.: 3551179). Take at least 400 µl (≤ 1000 µl) with a 1000 µl tip and transfer in one well of the filter plate (Ref.: 3551179), exclude the first 6 positions (from A1 to F1). Place a plastic sealing film on top the filter plate. Set the vacuum gauge of the pump (Ref.: 3590350) to 25.4 cm Hg (± 2.5%). Switch the pump on and check the gauge for correct vacuum, then open manifold valve for 1 minute ± 6 seconds. Close the valve, switch off the pump and release the vacuum from the manifold.

- Centrifugation technique:
  Take at least 400 µl (≤ 1000 µl) with a 1000 µl tip and transfer in one well of the filter plate (Ref.: 3551179) priorly fitted on a Deepwell plate (Ref.: 3590132) (the master plate), exclude the first 6 positions (from A1 to F1). Place a plastic sealing film on top the filter plate.
  Centrifuge the complete system (filter plate and Deepwell plate) for 1 min at 500 g. Taking care to keep the filtration plate securely in position on the Deepwell plate.
  Note: Centrifuge must be equipped with Deepwell microplate rotor (Ref.: 3590136), for 5804R Eppendorf centrifuge (Ref.: 3591396).

After either technique discard the filter plate and transfer 250 µl of filtered samples into another Deepwell (the purification plate) for the manual protocol or directly place the master plate on board the NSP (refer to the TeSeE™ NSP operator's manual).

Note: At this stage, grinding tubes after homogenisation, micro test-tubes and Deepwell plate after sample transfer can be stored, closed:

sNo	At room temperature (+18°C to +30°C) for 8 hours	At +2°C to 8°C (in ice or refrigerator) for 15 hours	At -20°C for 1 year*
Grinding tubes and micro test-tubes	Yes	Yes	Yes
Deepwell plate	Yes	Ye	

* 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 inversion before use.

4. PK treatment:
Distribute 250 µl (± 10%) of reconstituted proteinase K solution [see paragraph 2.4] into each micro-tube or Purification plate well. Do not exceed intervals of 5 minutes for distribution of reconstituted proteinase K between the first and the last sample. Immediately homogenise closed tubes or Deepwell sealed with aluminium film 10 times by inversion. Do not exceed 2 minutes between the homogenization and the incubation at 37°C.
Incubate at 37°C ± 2°C in a heating block incubator for 10 ± 1 minute.
Note: If using Deepwell, heating block must be equipped with a Deepwell rack adaptor for heating block (Ref.: 3590134).

5. Precipitation of PrP^{Sc} with reagent B:
Remove the micro test-tubes or Deepwell plate from the heating block incubator. Open the tubes and distribute 250 µl (± 10%) of reagent B into all micro test-tubes or Deepwell wells. Observe the same order of distribution as described in step 4. Do not exceed intervals of 2 minutes between the exit of the incubator and the homogenization. Homogenization is performed under the same conditions as in step 4.

6. Concentration of the PrP^{Sc} (centrifugation):
Within 30 minutes, after reagent B distribution and mixing : centrifuge the micro test tubes or purification plate as follows:

Centrifugation	Micro test-tubes		Deepwell plate
Speed (g)	20 000	15 000	2 000
Time (mm)	5	7	10
Temperature (°C)	20	20	4

Note: For Deepwell plate allow a 5 minute delay at 37°C or a 10 minute delay at room temperature (+18°C to +30°C) before centrifugation.

7. Sample clarifying:
Discard the supernatant by inverting the micro test-tubes over a waste container. Dry the micro test-tubes by inverting onto absorbent paper for 5 minutes.
Or load the Deepwell plate on DW40 unit (Ref.: 3590137). Select «TSE DW» program and select number of strips to be performed. Deepwell plate wells must be dried at the end of the DW40 process, by inverting the plate on absorbent paper for 5 minutes.
Distribute 25 µl (± 10%) of reagent C into all micro test-tubes or Deepwell wells.
Do not exceed an interval of 10 minutes between the end of the drying operation and distribution of buffer C.
Incubate immediately for 5 ± 1 minute at 100°C ± 5°C. Do not exceed 2 minutes between the reagent C distribution and the beginning of the incubation. Do not seal the Deepwell plate during incubation.
Note: If using Deepwell, heating block must be equipped with a Deepwell rack adaptor for heating block (Ref.: 3590134).
Remove the micro test-tubes or the Deepwell from the incubator, and homogenate the tubes with a vortex (5 ± 2 seconds).
Samples in micro test-tubes or Deepwell can be stored for 5 hours at +2°C to +8°C or frozen for 72 hours at -20°C. Frozen samples must be thawed at room temperature (+18°C to +30°C) and homogenized with a vortex (5 ± 2 seconds).

Purified samples must be diluted with 125 µl (± 10%) of reagent R6. Diluted samples must be homogenized with vortex (5 sec. ± 2 sec.) just before distribution into the plate (R1).

1. Remove the microplate rack and the required number of rows (R1) from the protective packaging. Replace the unused rows with the desiccated bag in the microplate sachet and hermetically close it.

2. Prepare the positive control (R4), as described in chapter 3.4.2.

3. For each series of tests and every single plate, distribute 100 µl (± 10%) of control/sample into wells in the following order:
   - Wells A1, B1, C1, D1: negative control (R3)
   - Wells E1, F1: positive control (R4)
   - Wells G1, H1, etc… : sample diluted with reagent (R6)
   Samples are performed in singulate.

4. Cover with adhesive film and incubate for 30 mn ± 2 mn at 37°C ± 2°C.

5. Prepare wash solution (R2).

6. Prepare conjugate solution (R7).

7. Remove the adhesive film, perform 3 wash cycles.
   Optimal washing conditions are obtained with PW40, PW41 or 1575 Bio-Rad plate washers with program TSE 3.
   Do not let the microplate stand for more than 5 minutes after the last wash cycle. Dry by inversion on absorbent paper before the following step.

8. Distribute 100 µl (± 10%) of conjugate solution (R7) into each well.

9. Cover with adhesive film and incubate 30 mn ± 2 mn at +2°C to +8°C.

10. Prepare the enzymatic revelation solution (R8+R9).

11. Remove the adhesive film, perform 5 wash cycles.
    Optimal washing conditions are obtained with PW40, PW41 or 1575 Bio-Rad plate washers with program TSE 5.
    Do not let the microplate stand for more than 5 minutes after the last wash cycle. Dry by inversion on absorbent paper before the following step.

12. Distribute 100 µl (± 10%) of revelation solution (R8+R9) into each well and incubate the plate in darkness and at room temperature (+18°C to +30°C) for 30 mn ± 2 mn. Do not use adhesive film during this incubation.

13. Add 100 µl (± 10%) of stop solution (R10) to each well according to the same sequence and same distribution rate as for the revelation solution.

14. Thoroughly wipe the bottom of the plate and determine the optical density at 450 nm - 620 nm (bichromatism mode) within 30 minutes after stopping the reaction (the rows must always be protected from light before reading).

Microplate washer parameters

NAME: TSE 3

EDIT mode function	PLATE	Manifold	STRIPS	Met. (Method)	MODE	CROS SW ASP.	ASP. TIME	VOLUME	OVER FLOW	LIQUID	FLOW	BOT. WASH NUMBER	BOTTOM TIME	BOT. ASP. NUMBER	SHAKE TIME	Nr OF CYCLES	SOAKING	MET. INTER	Nr OF KITS	KIT INTER
Main parameter	Flat 01 (PW40/PW41) Flat 03 (1575)	1*8 (PW40/1575) 2*8 (PW41)	1,2,3,4, 5,6,7,8,9, 10,11,12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Method 1	-	-	-	WASH	Plate	Yes	0,3	800	2,5	W1	0 (PW40/1575) 5 (PW41)	-	-	-	-	3	30 (PW41) 45 (PW40/1575)	0	-	-
Method 2	-	-	-	BOTTOM ASP.	Plate	Yes	0,3	-	-	-	-	-	-	1	-	1	0	-	-	-

NAME: TSE 5

EDIT mode function	PLATE	Manifold	STRIPS	Met. (Method)	MODE	CROS SW ASP.	ASP. TIME	VOLUME	OVER FLOW	LIQUID	FLOW	BOT. WASH NUMBER	BOTTOM TIME	BOT. ASP. NUMBER	SHAKE TIME	Nr OF CYCLES	SOAKING	MET. INTER	Nr OF KITS	KIT INTER
Main parameter	Flat 01 (PW40/PW41) Flat 03 (1575)	1*8 (PW40/1575) 2*8 (PW41)	1,2,3,4, 5,6,7,8,9, 10,11,12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Method 1	-	-	-	WASH	Plate	Yes	0,3	800	2,5	W1	0 (PW40/1575) 5 (PW41)	-	-	-	-	5	30 (PW41) 45 (PW40/1575)	0	-	-
Method 2 -	-	-	-	BOTTOM ASP	Plate	Yes	0,3	-	-	-	-	-	-	1	-	1	0	-	-	

PLATE NAME: FLAT 01 (PW40/PW41) - FLAT 03 (1575)

BOT: SHAPE	ASP. HOR. POS.	CENTERING	ASP. VERT. POS.	BOT. VERT. POS.	B.W. VERT. POS.	HORIZONTAL SPEED	VERTICAL SPEED	ASP. DOWNW. SPEED	DISP. UPW. SPEED	BOT. DOWNW. SPEED	BOT. UPWARD SPEED	SHAKING AMPLITUDE	SHAKING SPEED
Flat	1,4	0,3	13,5	9,5	9,5	6	8	6	9	6	9	1	9

2-7 Calculation and Interpretation of the Results

1) Calculation of the mean optical density (OD) of the negative control

$\overline{OD\ R3}$ = mean of the four OD of R3 wells

2) Calculation of the cut-off value

The cut-off value is equal to: $\overline{OD\ R3}$ + 0.210

Example:

$\overline{OD\ R3}$ = 0.020

Cut-off value = 0.020 + 0.210 = 0.230

3) Condition of validation of the test

- Negative control (R3):

a) Validation of the individual negative control values:

The optical density of each individual negative control must be lower than 0.150.

However, a maximum of one individual aberrant value can be eliminated when its optical density is higher or equal to 0.150.

The test must be repeated if more than one of the negative control lies outside of this limit.

b) Homogeneity of the negative control values:

Calculate the mean of the negative controls with the individual remaining values.

Values higher than the mean of the negative controls + 40% ($\overline{OD\ R3}$ + 40%) must be eliminated.

- If one individual value is eliminated in a), one additional value can be eliminated in b).
- If no negative control value is eliminated in a), two values maximum can be eliminated in b).

The test must be repeated if more than two values of the negative control are eliminated [criteria a)+b)].

- Positive control (R4):

The mean of the positive control optical densities (R4 ODs) must be higher or equal than 1.000.

The test must be repeated if the mean of the positive control optical densities (R4 ODs) is strictly lower than this limit.

4) Interpretation of the results

Samples with an optical density lower than the cut-off value are considered to be negative according to the TeSeE™ SAP Kit instructions.

However, results situated just below the cut-off value (cut-off value - 10%) must be interpreted cautiously, and the corresponding samples should be retested in duplicate, starting from the original homogenate.

Samples with an optical density greater than or equal to the cut-off value are considered to be initially reactive according to the TeSeE™ SAP Kit instructions and should be retested in duplicate, starting from the original homogenate, before the final interpretation.

After repeating the test, the sample is considered to be positive according to the TeSeE™ SAP Kit instructions when at least one of the 2 measurements is positive (greater than or equal to the cut-off value). The sample is considered to be negative according to the TeSeE™ SAP Kit instructions when these two values are less than the cut-off value.

Samples retested in duplicate and found to be negative according to the TeSeE™ SAP Kit instructions, but for which one of the 2 values is close to the cut-off value (cut-off value - 10%) must be interpreted cautiously.

2-8 Limits of the Test

Difficulties can be encountered during the grinding step when using dehydrated samples or peripheral tissues. If necessary, the grinding step (step No.2 of the procedure) may need to be repeated several times for this type of sample.

A negative result means that the test sample does not contain any PrP^{Sc} detectable by the TeSeE™ SAP Combi Kits. However, as very low levels of PrP^{Sc} may not be detected, such a negative result does not exclude the possibility of infection.
Any sample with a reproducible positive result according to the test interpretation criteria must be confirmed in accordance with the countries national reference laboratory for TSEs or community reference laboratory in exceptional circumstances.

3 - MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled or ultrapure water.
- 20 000 ppm sodium hypochlorite (final concentration) and 1 M sodium hydroxide (final concentration).
- Absorbent paper.
- Disposable gloves.
- Protective glasses or mask with visor.

Purification step:

- 2 ml polypropylene micro test-tubes with caps and appropriate tube rack.
- Automatic or semiautomatic adjustable pipettes able to distribute volumes between 20 µl and 500 µl.
- Tissue homogenizer: Ribolyser®, TeSeE™ PRECESS 24™ or TeSeE™ PRECESS 48™.*
- Centrifuge* adapted to micro test-tubes.
- One micro test-tube heating block* thermostated at 37°C ± 2°C and one micro test-tube heating block* thermostated at 100°C ± 5°C.

For the semi-automatic purification of the sample: TeSeE™ NSP System.

Detection step:

- Automatic or semiautomatic adjustable or fixed pipettes able to distribute 50 µl, 100 µl, 200 µl and 1000 µl.
- 10 ml, 20 ml, 100 ml graduated test tubes.
- Contaminated waste containers.
- Microplate incubator thermostated at 37°C ± 2°C.
- Refrigerated chamber at +2°C to +8°C.
- Automatic or semiautomatic microplate washing system.*
- Microplate reading apparatus* (equipped with 450 nm and 620 nm filters).
- Microplate system* for the automation of the assay protocol stages. The performances of the system must conform with the requirements of the test protocol.

** Contact Bio-Rad for the list of available instruments.*

4 - PRECAUTIONS

The quality of the results depends on compliance with the following good laboratory practices:

- Reagents must be stored at +2°C to +8°C.
- Do not use reagents whose shelf-life has expired.
- Do not use the reconstituted and stored at room temperature (+18°C to +30°C) proteinase K over 6 hours.
- Do not mix reagents derived from different batches of the TeSeE™ SAP Kits during the same manipulation, with the exception of generic reagents: wash solution (R2), sample diluent (R6), peroxidase substrate buffer (R8), chromogen (R9), stop solution (R10), grinding tubes, reagent A and reagent B.
- Wash solution (R2), sample diluent (R6), peroxidase substrate buffer (R8), chromogen (R9), stop solution (R10) and grinding tubes can be used with all kits from the TeSeE™ product line (TeSeE™, TeSeE™ SAP and TeSeE™ sheep/goat assays).
- Allow the reagents 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, alkalis, aldehydes) or dust, which could alter the enzymatic activity of the conjugate.
- Only use polypropylene tubes.
- Use perfectly washed glassware, rinsed in distilled water, or preferably disposable material.
- Do not let the microplate more than 5 minutes between the end of washing and distribution of the reagents.
- The enzymatic reaction is very sensitive to all metals or metallic ions. Consequently, no metallic element must enter in contact with the various solutions containing the conjugate or the substrate.
- The revelation solution (substrate buffer + chromogen) must be colorless. The appearance of a colour few minutes after reconstitution indicates that the reagent cannot be used and must be replaced. The revelation solution should preferably be prepared with disposable plastic containers and distribution material or glassware previously washed in 1 N hydrochloric acid, rinsed in distilled water and dried. Store this solution protected from light.
- Use a new pipette tip for each sample.
- Washing of the wells is an essential step of the procedure: respect the recommended number of washing cycles and ensure that all wells are completely filled, then completely emptied. Inadequate washing can give incorrect results.
- Never use the same container and pipette to distribute the conjugate and the revelation solution.

5 - HYGIENE AND SAFETY INSTRUCTIONS

Generally, hygiene conditions, biosafety mesures and good laboratory practices must be in agreement with recommendation of regular authorities of the country.

- 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 with the mouth.
- Use polypropylene containers to avoid any wounds with 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 special container for contaminated wastes.
- Samples, material and contaminated products must be eliminated after decontamination:
  - either by soaking in 1 M sodium hydroxide (final concentration) for 1 hour at room temperature (+18°C to +30°C),

- or by soaking in 20 000 ppm sodium hypochlorite solution for 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 sodium hypochlorite solution 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.
- Avoid any contact of the substrate buffer, chromogen and stopping solution with the skin and mucous membranes.
- 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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Sample Syringe

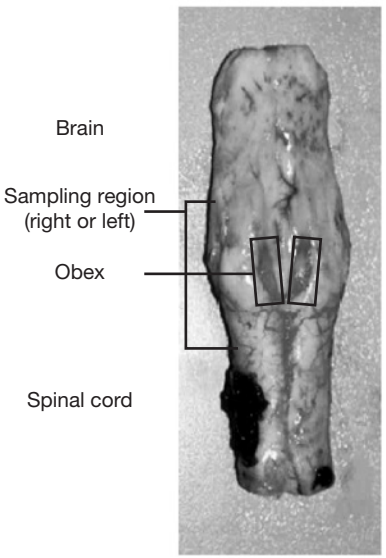
REF 3551175

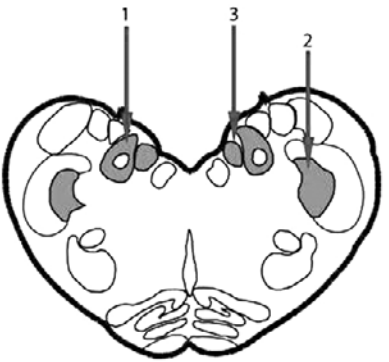
SAMPLING METHOD FOR Bio-Rad TSE SCREENING ASSAYS

TABLE OF CONTENTS

1 - GENERAL INFORMATION

The Bio-Rad TSE screening assays are performed on a sample of 350 ± 40 mg of central nervous tissues (CNS). The specific anatomical region for detecting PrP^{Sc} in infected animals is the brain stem, more precisely in the area of the vagal nerve nucleus, in the obex region. This is the area of the brainstem where PrP^{Sc} is most concentrated.



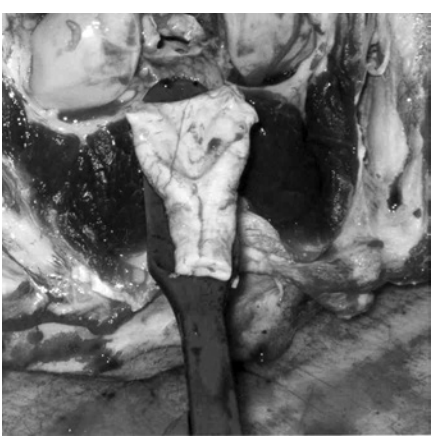


Cross section of the brain stem at the level of the obex identifying the key target sites for diagnosis by histopathology and immunohistochemistry in BSE (nucleus of the solitary tract [1] and the nucleus of the trigeminal tract V [2]) and scrapie (dorsal nucleus of the vagus). [3]).

(Source: OIE - Manual of Diagnostic Tests and Vaccines for Terrestrial Animals)

1-1 Sample collection at the abattoir

The brain stem is easily and quickly collected with an appropriate tool or sample collection spoon, via the occipital foramen, without opening the cranial cavity.



Sample collection with the Bio-Rad collection spoon

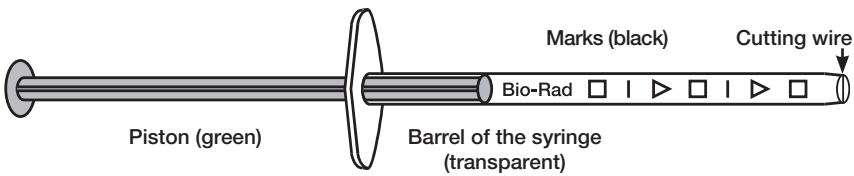
1-2 Sampling procedure at the laboratory

The whole brain stem sample is sent to the testing laboratory ensuring that appropriate bio-safety measures recommended by the regulatory authorities of the particular country are followed. In the laboratory, the appropriate amount of cerebral material is cut (scalpel blade,…) from the obex region or collected with the **Bio-Rad sample syringe (Ref: 3551175)** which makes it possible to sample the required amount of the appropriate area quickly and safely, without any risk of sharps injuries.

The following describes the procedure to effectively collect the sample from the obex region using the Bio-Rad sample syringe, without damaging the tissue.

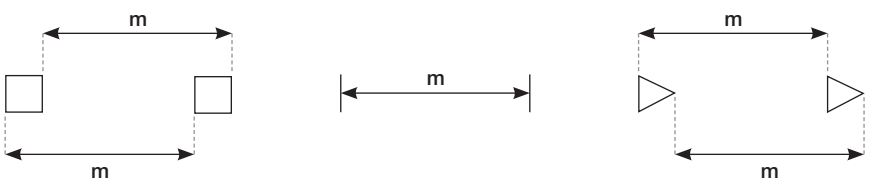
2 - Bio-Rad SAMPLE SYRINGE

The Bio-Rad sample syringe consists of a green piston and a transparent syringe barrel. The syringe barrel is labelled with a series of geometric shapes. (□ ▷ I)



3 - SAMPLE MASS REQUIRED FOR THE TEST

The sample mass should occupy the space between two symbols of the same shape which corresponds to a mass (m) of 350 +/- 40 mg.



4 - OPERATING PROCEDURE

- Take a sample syringe and pull out the green piston to approximately 1 cm from its home position then push home again.

- Firmly grasp the brain stem in one hand, using a disposable wrapper (plastic bag, glove, etc.) in order to avoid possible cross-sample contamination. The end of the brain stem should remain accessible. If the brainstem received has a cord too long, the user should trim it. Samplers should received proper training regarding the précise location of the targetted area.

- Use the other hand to position the open end of the sampling syringe on the right or left side of the caudal end of the brain stem.

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

- Insert the syringe barrel gradually into the brain stem whilst holding the green piston stationary (relative to the brain stem).

Note: While collecting the sample from the obex region, take care that the syringe barrel remains within the selected side of the brain stem.

- Stop this movement when the top of the syringe barrel has reached the upper limit of the sampling zone.

- Cut the sample core by twisting the syringe barrel through one complete turn.

- Slowly remove the sample syringe from the brain stem, taking care not to damage surrounding tissues. The remaining brain stem can be placed in its original sample container.

- Check whether there are any air gaps in the core sample collected. If needed, compress the sample core by closing the top of the syringe barrel and pushing the green piston until the air gaps have been eliminated. At the same time ensure that the tissue nearest the opening of the syringe barrel is retained.

- **Holding the top of the syringe barrel still, move the green piston to the nearest symbol.**

- Check that the sample core covers at least one zone corresponding to “m”, as described in the previous section of this document (sample mass required for the test).

- Take a grinding tube and remove the lid, with the sample syringe carefully depress the green piston to the next identical symbol to ensure that the correct mass of tissue (“m”) is dispensed in the grinding tube. Remember that you must move the piston to the corresponding position of the next symbol as indicated in “Sample mass required for the test”.

- Cut the sample core by gripping the top of the sample syringe against the inner edge of the grinding tube.

- Samples of extremely bad quality should be either disected or if very autolysed pipetted up.

- The unused part of the sample core can be stored by placing the sample syringe in the original container with the remaining piece of brain stem.

5 - PRECAUTIONS/ADVICE

As for any pipetting device, Bio-Rad recommends that operators using the sample syringe should be periodically monitored, for a representative statistical population of samples taken, so ensuring that sample weights are within range.

The sample syringes are to be used only once, and then discarded in order to prevent any cross-sample contamination.
The sample must be taken with all due precautions in order to ensure that risk of contamination for operators is minimized.
The syringes used are to be discarded after being decontaminated (see Health & Safety instructions).
If the sample core does not fill the entire syringe barrel despite carrying out the procedure correctly, it is advisable to weigh the sample.

6 - HEALTH & SAFETY PROCEDURES

The hygiene conditions, bio-safety measures and good laboratory practices must comply with the guidelines of the regulatory authorities in the country.
The sample syringe is intended for use in "*in-vitro*" diagnostic procedures only.

Wear disposable gloves when handling reagents and samples, and wash your hands thoroughly after handling them.

Any equipment that has come into direct contact with the samples must be considered to have been contaminated.

Contaminated surfaces must be cleaned with 20 000 ppm sodium hypochlorite solution. When the contaminating liquid is an acid, contaminated surfaces must first be neutralized with sodium hydroxide before using sodium hypochlorite. 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, equipment and contaminated products must be discarded after decontamination using one of the following methods:

- by soaking in 1 M sodium hydroxide (final concentration) for 1 hour at room temperature (+18°C to +30°C).
- by soaking in 20 000 ppm sodium hypochlorite solution for 1 hour at room temperature (+18°C to +30°C).
- by autoclaving at a temperature of at least 134°C for a minimum of 18 minutes, at 3 bar pressure.
- **Note: never autoclave solutions containing bleach.**

All operations involved in Transmissible Spongiform Encephalopathy (TSE) screening tests are subject to local safety guidelines and must be performed in an isolated, limited and controlled-access laboratory devoted exclusively to this activity. A laboratory coat or boiler suit, overshoes, gloves (two pairs), 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 TSE agents or prions, and the validated methods of decontamination for unconventional agents. Bio-safety measures must comply with the Guidelines of the regulatory authorities of the country concerned.

(BG) • Този продукт съдържа човешки или животински компоненти. Бъдете внимателни при работа с него.

(CN) • 本产品包含人/动物成分，请小心处理。

(CN) Traditional • 本產品包含人/動物成分，請小心處理。

(CZ) • Tento výrobek obsahuje lidské nebo zvířecí komponenty. Zacházejte s ním opatrně.

(DE) • Dieses Produkt enthält Bestandteile menschlichen oder tierischen Ursprungs. Vorsichtig handhaben.

(DK) • Dette produkt indeholder humane og animalske komponenter. Skal behandles med forsigtighed.

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

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

(ES) • Este producto contiene componentes humanos o animales. Manejar con cuidado.

(FI) • Tässä tuotteessa on ihmisestä tai eläimistä peräisin olevia osia. Käsittele varovasti.

(FR) • Ce produit contient des composants d'origine humaine ou animale. Manipuler avec précaution.

(GR) • Αυτό το προϊόν περιέχει ανθρώπινα ή ζωικά στοιχεία. Χειριστείτε το με προσοχή.

(HR) • Ovaj proizvod sadrži ljudske ili životinjske sastojke. Pažljivo rukovati.

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

(IT) • Questo prodotto contiene componenti umane o animali. Maneggiare con cura.

(JP) • 本製品にはヒトまたは動物由来の構成成分が含まれます。取り扱いにご注意下さい。

(KR) • 본 제품은 사람 또는 동물유래의 성분이 포함되어 있습니다. 취급에 주의하시기 바랍니다.

(LT) • Šiame produkte yra žmogiškosios arba gyvūninės kilmės sudėtinių dalių. Elgtis atsargiai.

(MT) • Dan il-prodott fih komponenti umani jew tal-annimali. Uża b'attenzjoni.

(NL) • Dit product bevat menselijke of dierlijke bestanddelen. Breekbaar.

(NO) • Dette produktet inneholder humane eller animalske komponenter. Håndteres med forsiktighet.

(PL) • Niniejszy produkt zawiera składniki pochodzenia ludzkiego lub zwierzęcego. Należy obchodzić się z nim ostrożnie.

(PT) • Este medicamento contém componentes de origem humana ou animal. Manuseie com cuidado.

(RO) • Acest produs conține materiale de origine umană sau animală. Manevrați-l cu grijă.

(SE) • Denna produkt innehåller beståndsdelar från människa eller djur. Hantera produkten varsamt.

(SI) • Izdelek vsebuje človeške ali živalske sestavine. Rokujte previdno.

(SK) • Tento výrobok obsahuje ľudské alebo zvieracie zložky. Narábajte s ním opatrne.

H226 - H314 - H317 - H334 - H412
P210 - P261 - P280
P305+P351+P338 - P302+P352
P333+P313 - P501

(BG)
опасно
Запалими течност и пари. Причинява тежки изгаряния на кожата и сериозно увреждане на очите. Може да причини алергична кожна реакция. 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. При поява на кожно дразнене или обрив на кожата: Потърсете медицински съвет/помощ. 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. 引起严重的皮肤灼伤和眼睛损伤. 可能引起皮肤过敏性反应. 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。. 如发生皮肤刺激或皮疹：求医/就诊。. 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。

(CN) Traditional
危險
易燃液體和蒸氣. 引起嚴重的皮膚灼傷和眼睛損傷 可能引起皮膚過敏性反應. 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。. 如發生皮膚刺激或皮疹：求醫/就診。. 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 těžké poleptání kůže a poškození očí. Může vyvolat alergickou kožní reakci. 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. Při podráždění kůže nebo vyrážce: Vyhledejte lékařskou pomoc/ošetření. Zabraňte uvolnění do životního prostředí. Obsah/nádobu likvidujte v souladu s místními/regionálními/národními/mezinárodními předpisy.

(DE)
Gefahr
Flüssigkeit und Dampf entzündbar. Verursacht schwere Verätzungen der Haut und schwere Augenschäden. Kann allergische Hautreaktionen verursachen. 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. Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ärztliche Hilfe hinzuziehen. Freisetzung in die Umwelt vermeiden. Entsorgung des Inhalts / des Behälters gemäß den örtlichen / regionalen / nationalen/ internationalen Vorschriften.

(DK)
Fare
Brandfarlig væske og damp. Forårsager svære forbrændinger af huden og øjenskader. Kan forårsage allergisk hudreaktion. 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. Ved hudirritation eller udslet: Søg lægehjælp. Undgå udledning til miljøet. Bortskaffelse af indholdet/beholderen i henhold til de lokale/regionale/nationale/internationale forskrifter.

(EE)
Ettevaatust
Tuleohtlik vedelik ja aur. Põhjustab rasket nahasöövitust ja silmakahjustusi. Võib põhjustada allergilist nahareaktsiooni. 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.

Nahaärrituse või _obe korral: pöörduda arsti poole. Vältida sattumist keskkonda. Sisu/konteineri käitlus vastavuses kohalike/regionaalsete/rahvuslike/rahvusvaheliste nõuetega.

(EN)
Danger
Flammable liquid and vapour. Causes severe skin burns and eye damage. May cause an allergic skin reaction. 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. If skin irritation or rash occurs: Get medical advice/attention. Avoid release to the environment. Dispose of contents/container in accordance with local/regional/national/international regulations.

(ES)
Peligro
Líquidos y vapores inflamables. Provoca quemaduras graves en la piel y lesiones oculares graves. Puede provocar una reacción alérgica en la piel. 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. En caso de irritación o erupción cutánea: Consultar a un médico. 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. Voimakkaasti ihoa syövyttävää ja silmiä vaurioittavaa. Voi aiheuttaa allergisen ihoreaktion. 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. Jos ilmenee ihoärsytystä tai ihottumaa: Hakeudu lääkäriin. Vältettävä päästämistä ympäristöön. Säilytä säiliö(t) noudattaen paikallisia/alueellisia/kansallisia/kansainvälisiä määräyksiä.

(FR)
Danger
Liquide et vapeurs inflammables. Provoque des brûlures de la peau et des lésions oculaires graves. Peut provoquer une allergie 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. En cas d'irritation ou d'éruption cutanée: consulter un médecin. 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 σοβαρά δερματικά εγκαύματα και οφθαλμικές βλάβες. Μπορεί να προκαλέσει αλλεργική δερματική αντίδραση. 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. Εάν παρατηρηθεί ερεθισμός του δέρματος ή εμφανιστεί εξάνθημα: Συμβουλευθείτε/Επισκεφθείτεγιατρό. 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 opekline kože i ozljede oka. Može izazvati alergijsku reakciju na koži. 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 ispiranje. U SLUČAJU DODIRA S KOŽOM: oprati velikom količinom sapuna i vode. U slučaju nadražaja ili osipa na koži: zatražiti savjet/pomoć liječnika. Izbjegavati ispuštanje u okoliš. Odložite sadržaje /spremnike u skladu s lokalnim/regionalnim/nacionalni/međunarodnim odredbama.

(HU)
Veszély
Tűzveszélyes folyadék és gőz. Smarkiai nudegina odą ir pažeidžia akis. Allergiás bőrreakciót válthat ki. 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. Bőrirritáció vagy kiütések megjelenése esetén: orvosi ellátást kell kérni. 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 ustioni cutanee e gravi lesioni oculari. Può provocare una reazione allergica 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. In caso di irritazione o eruzione della pelle: consultare un medico. 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. 重篤な皮膚の薬傷及び眼の損傷 アレルギー性皮膚反応を起こすおそれ. 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。. 皮膚刺激又は発しん（疹）が生じた場合：医師の診断／手当てを受けること。. 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. 피부에 심한 화상과 눈에 손상을 일으킴 알레르기성 피부 반응을 일으킬 수 있음. 흡입시 알레르기성 반응, 천식 또는 호흡 곤란을 일으킬 수 있음. 장기적인 영향에 의해 수생생물에게 유해함.
열·스파크·화염·고열로부터 멀리하시오 – 금연. (본진·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. 피부자극성 또는 홍반이 나타나면 의학적인 조치·조언을 구하시오. 환경으로 배출하지 마시오. 현지/지역/국가/국제규정에 따라서 내용물/용기 노출.

(LT)
Pavojinga
Degūs skystis ir garai. Smarkiai nudegina odą ir pažeidžia akis. Gali sukelti alerginę odos reakciją. 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. Jeigu sudirginama oda arba ją išberia: kreiptis į gydytoją. Saugoti, kad nepatektų į aplinką. Turinį/talpą išpilti (išmesti) - šalinti pagal vietines / regionines / nacionalines / tarptautines taisykles.

(NL)
Gevaar
Ontvlambare vloeistof en damp. Veroorzaakt ernstige brandwonden en oogletsel. Kan een allergische huidreactie veroorzaken. 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. Bij huidirritatie of uitslag: een arts raadplegen. 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 hudforbrenninger og øyeskader. Kan forårsake allergiske hudreaksjoner. 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. Ved hudirritasjon eller -utslett: Kontakt / tilkall lege. 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 oparzenia skóry oraz uszkodzenia oczu . Może powodować reakcję alergiczną skóry. 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. W przypadku wystąpienia podrażnienia skóry lub wysypki: Zasięgnąć porady/zgłosić się pod opiekę lekarza. Unikać uwolnienia do środowiska. Zawartość / pojemnik usuwać zgodnie z przepisami miejscowymi / regionalnymi / narodowymi / międzynarodowymi.

(PT)
Perigo
Líquido e vapor inflamáveis. Provoca queimaduras na pele e lesões oculares graves. Pode provocar uma reacção alérgica 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. Em caso de irritação ou erupção cutânea: consulte um médico. 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 arsuri grave ale pielii şi lezarea ochilor. Poate provoca o reacţie alergică a 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 inspiraţi praful/fumul/gazul/ceaţa/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. În caz de iritare a pielii sau de erupţie cutanată: consultaţi medicul. Evitaţi dispersarea în mediu. Aruncaţi conţinutul/containerul în acord cu regulamentele locale/regionale/naţionale/internaţionale.

(SE)
Fara
Brandfarlig vätska och ånga. Orsakar allvarliga frätskador på hud och ögon. Kan orsaka allergisk hudreaktion. 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. Vid hudirritation eller utslag: Sök läkarhjälp. Undvik utsläpp till miljön. Innehållet / behållaren avfallshanteras enligt lokala / regionala / nationella / internationella föreskrifter.

SI)
Nevarno
Vnetljiva tekočina in hlapi. Povzroča hude opekline kože in poškodbe oči. Lahko povzroči alergijski odziv 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. Če nastopi draženje kože ali se pojavi izpuščaj: poiščite zdravniško pomoč/oskrbo. Preprečiti sproščanje v okolje. Vsebino/vsebnik odstranite v skladu z lokalnimi/regionalnimi/narodnimi/mednarodnimi predpisi.

(SK)
Nebezpečenstvo
Horľavá kvapalina a pary. Provoacă arsuri grave ale pielii şi lezarea ochilor. Môže vyvolať alergickú kožnú reakciu. 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. Ak sa prejaví podráždenie pokožky alebo sa vytvoria vyrážky: vyhľadajte lekársku pomoc/starostlivosť. Zabráňte uvoľneniu do životného prostredia. Zneškodnenie obsahu/obalu v súlade s miestnymi/oblastnými/národnými/medzinárodnými nariadeniami.

Bio-Rad
3, boulevard Raymond Poincaré
92430 Marnes-la-Coquette - France
Tel.: +33 (0)1 47 95 60 00
Fax: +33 (0)1 47 41 91 33
www.bio-rad.com

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