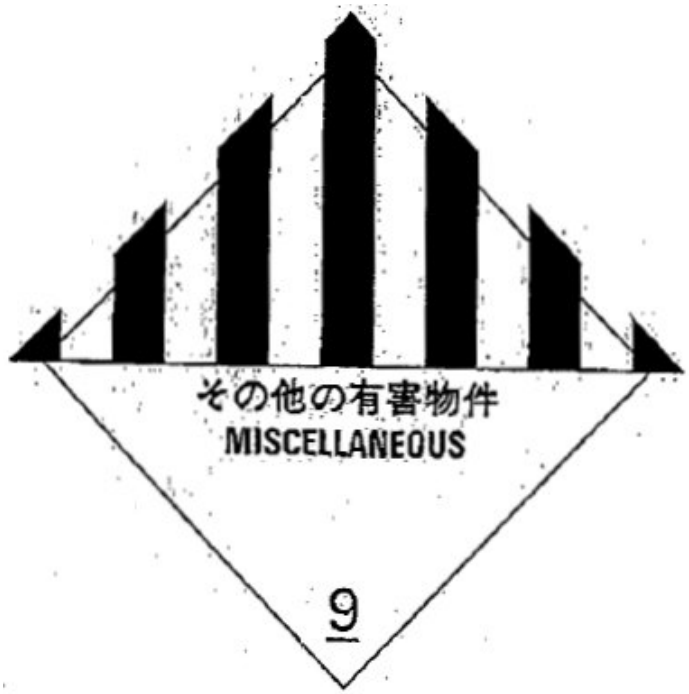
(Separate Form 1-3)

1. Transportation Permit Label (Classification No.: 6.2)

2. Transportation Permit Label (Classification No.: 9)



(Separate Form 1-4)

(Air Transportation)

Declaration of Hazardous Substance in Postal Matter (Bovine tissue, etc.)

The item name and quantities, etc. described below regarding this postal package are entirely accurate. The material is stored in a container complying with UN specifications, and packaged, labeled, etc. according to the Aviation Law and its relevant regulations. This postal package is within the carrying limits of aircraft and is in suitable condition for air transport.

Date of Form Filling		D/M/Y:	
Item		Bovine tissue, etc.	
	UN2814 UN2900	Highly infectious substance transmissible to humans and animals (Liquid)	(Note 1) mL
	UN2814 UN2900	Highly infectious substance transmissible to humans and animals (Solid)	(Note 2) g
	UN1845	Dry ice	kg
	Stored in a container complying with UN specifications, packed with dry ice and further packaged in another container or such.		

Sender
Local Authority:
Laboratory :
Address:
Phone No.:
Name: Livestock Inspector (Veterinarian)

Recipient
Name of Laboratory:
Address:
Phone No.:
Name:

Margin for air carrier use

Note 1: The limit to contents stored within a single container is less than 1000 mL for liquids.
Note 2: The limit to contents stored within a single container is less than 50 g for solids.

(Separate Form 1-4)
(Example)

(Air Transportation)

Declaration of Hazardous Substance in Postal Matter (Bovine tissue, etc.)

The item name and quantities, etc. described below regarding this postal package are entirely accurate. The material is stored in a container complying with UN specifications, and packaged, labeled, etc. according to the Aviation Law and its relevant regulations. This postal package is within the carrying limits of aircraft and is in suitable condition for air transport.

Date of Form Filling		D/M/Y: 30/10/2002	
Item		Bovine tissue, etc.	
	UN2814 UN2900	Highly infectious substance transmissible to humans and animals (Liquid)	(Note 1) mL
√	UN2814 UN2900	Highly infectious substance transmissible to humans and animals (Solid)	(Note 2) 40 g
√	UN1845	Dry ice	3 kg
√	Stored in a container complying with UN specifications, packed with dry ice and further packaged in another container or such.		

Sender
Local Authority: XX Prefecture
Laboratory : XXXXX Meat Inspection Station
Address: 1-2-3, XXXX, XXX-city
Phone No.: XXX-XXX-XXXX
Name: Livestock Inspector (Veterinarian)
XXXXXXX

Recipient
Name of Laboratory: XXX Laboratory
Address: 3-2-1 XXX, XXX-city, XXX Prefecture, Postal code: XXX-XXXX
Phone No.: XXX-XXX-XXXX
Name: XXXXXXX

Margin for air carrier use

Note 1: The limit to contents stored within a single container is less than 1000 mL for liquids.
Note 2: The limit to contents stored within a single container is less than 50 g for solids.

(Separate Attachment 1-1)

Procedures for Platelia BSE

1. Sample Purification

(1) Preparation of reagents

Dilute the Proteinase K by 250-fold using the **Dissolver Solution** (**Reagent A**) in the BSE Purification Kit, in the required amounts depending on the number of samples to be treated (see table below).

(The diluted Proteinase K Solution may be stored for 4 hours at room temperature.)

No. of Samples	Volume of Reagent A	Volume of Proteinase K
2	2 mL	8 μL
10	6 mL	24 μL
18	10 mL	40 μL
26	14 mL	56 μL
34	18 mL	72 μL
42	22 mL	88 μL
50	26 mL	104 μL
58	30 mL	120 μL
66	34 mL	136 μL
74	38 mL	152 μL
82	42 mL	168 μL
90	46 mL	184 μL

(2) Purification of abnormal prion peptides

1) Collect and weigh **350 ± 40 mg** of the bovine obex region.
2) Place the weighed sample in the grinding tube.
3) Completely homogenize the sample in the grinding tube. (The sample thus homogenized may be stored for several weeks at -20°C, during which time it may be frozen/thawed only once.)
4) Collect 500 μL of the homogenated sample, taking care not to include solids, and transfer into a **2 mL microtube**, etc. (This sample may be stored for 8 hours at 2-8°C, or for several weeks at -20°C.)
5) Add 500 μL of the diluted **Proteinase K Solution** prepared in procedure 1. (1) above and mix well. In order to allow for uniform enzymatic activity, the procedure of adding the Proteinase K Solution must be completed swiftly within 5 minutes, or within 10 minutes if placed on ice.
6) When mixed well, immediately place in a hot water bath, incubator, or heat block, etc., and incubate for **10 ± 1 minutes at 37 ± 1°C**. The time interval between procedures 5) and 6) above shall be **less than 2 minutes**.
7) When incubation is complete, add 500 μL of **Reagent B** within 2 minutes (within 10 minutes if the tube is placed on ice) and mix the solution until it becomes a blue color overall. (The procedure for adding Reagent B shall be completed swiftly **within 5 minutes**, or within 10 minutes if placed on ice.)
8) Centrifuge the solution at **20000 × g for 5 minutes** or at **15000 × g for 7 minutes**.

9) After centrifuging, discard the supernatant **within 5 minutes**. In order to remove as much of the supernatant as possible, set the tube upside down on paper for 5 minutes, or dehydrate by aspiration for 5 minutes using an aspirator.
10) After discarding the supernatant, add 50 μL of **Reagent C1** to the microtube **within 10 minutes**. Do not mix by vortex.
11) Immediately place in hot water bath, incubator or heat block, and incubate for **5 ± 1 minutes at 100 ± 1°C**. The time interval between procedures 10) and 11) above shall be **less than 2 minutes**.
12) Remove the microtube from the incubator and mix well by vortex. (This sample may be stored for 5 hours at 2-8°C, or for several weeks at -20°C. Following any storage, it should be incubated for **5 ± 1 minutes at 100 ± 1°C** and mixed by vortex.)
13) Add 250 μL of the **Diluent** (**R6**) in the BSE Detection Kit and mix. (This sample may be stored for 5 hours at 2-8°C. After storing, mix well, then perform the following procedures.) After mixing, dispense the sample in the wells of the microplate of the Detection Kit. (To be continued in procedure 2. (2) 2).)

2. Sample Detection

(1) Preparation of reagents

1) Remove the reagents and solidifying microplate from the refrigerator prior to use and allow them to return to room temperature (20 ± 5°C).
2) Dilute the **Wash Concentrate** (**R2**) 10-fold using purified water and mix to make the **Wash Solution** (**R2'**). (May be stored for 2 weeks at 2-8°C)
3) Lightly tap the **Positive Control** (**R4**) bottle before opening, and then add 2 mL of purified water or Diluent (R6). Let stand for 1 minute, then mix gently to dissolve. (May be stored for 2 hours at 2-8°C, or for 6 months at -20°C after dispensing into adequate portions) When freezing, immediately dispense into microtubes, etc. after dissolving, and store at -20°C.
4) Immediately before use, dilute the **Enzyme-Labeled Antibody** (**R7**) 10-fold using the Wash Solution, and mix gently, to make the **Enzyme-Labeled Antibody Solution** (**R7'**). 1 mL of Enzyme-Labeled Antibody Solution (R7') is required per strip. (May be stored for 6 hours at 2-8°C)
5) In a container light-shielded with aluminum foil, etc., mix the **Substrate Buffer** (**R8**) and **Chromogen Liquid** (**R9**) in a ratio of 10:1, to make the **Chromogenic Substrate Solution** (**R8+R9**). 1 mL of Chromogenic Substrate Solution (R8+R9) is required per strip. (May be stored for 6 hours at room temperature. However, if it shows a blue color at the time of use, discard and prepare a fresh solution.)

(2) Detection of abnormal prions

1) Take the required number of strips from the microplate rack. (Return unused strips to the bag with the desiccant, and thoroughly expel any remaining air before resealing the bag. May be stored for 1 month at 2-8°C.)
2) Dispense the **Negative Control** (**R3**), **Positive Control** (**R4**) and the **sample** prepared using the BSE Purification Kit into the wells of the microplate as described below. A control must be provided on each plate when using multiple plates for screening, or for each run when using a single microplate for multiple

runs.

A1, B1, C1, D1: **Negative Control** (**R3**) 100 μL
E1, F1: **Positive Control** (**R4**) 100 μL
G1, H1: **Sample** 100 μL

3) Cover the microplate with sealing film and incubate for 75 ± 15 minutes at 37 ± 1°C using a heat block (preferable) or incubator.
4) Remove the sealing film and wash the plate with the Wash Solution (18-22°C). If using an automatic microplate washer, set an overflowing volume at 800 μL per well and run 3 wash cycles. When washing manually, remove the solution in the well, then fill with 350 μL of Wash Solution and remove, and repeat these steps 3-6 times (adjust the number of wash repetitions while observing the value). When washing is complete, completely remove the washing solution from the well by tapping it out on paper. Do not leave in this position for 5 minutes or longer.
5) Dispense 100 μL of the **Enzyme-Labeled Antibody Solution** (**R7'**) into each well.
6) Cover with sealing film, and incubate for 60 ± 5 minutes at 2-8°C.
7) Remove the sealing film and wash the plate with the Wash Solution (18-22°C). If using an automatic microplate washer, set an overflowing volume at 800 μL per well and run 5 wash cycles. When washing manually, remove the solution in the well, then fill with 350 μL of Wash Solution and remove, and repeat these steps 5-10 times (adjust the number of wash repetitions while observing the value). When washing is complete, completely remove the washing solution from the well by tapping it out on paper. Do not leave in this position for 5 minutes or longer.
8) Dispense 100 μL of the Chromogenic Substrate Solution (R8+R9) into the well, and after taking measures such as covering the plate with aluminum foil, incubate for 30 minutes at room temperature (18-22°C) in the dark, protected from light. Do not use film for incubation.
9) Dispense 100 μL of the **Reaction Stopper** (**R10**) into the wells.
10) Within 30 minutes after adding the Reaction Stopper, measure the OD using a microplate reader, at **main wavelength 450 nm** and **reference wavelength 620 nm**[1]. Avoid all light exposure prior to making the measurement.

[1] Any reference wavelength within the range of 600-700 nm will yield consistent results.

3. Interpretation

A cutoff value determined as follows is used for interpretation.

Cutoff value = (Mean absorption of 4 negative controls + Constant 0.210)
As the constant is periodically updated, the value indicated in the instructions of the Kit is to be used.
When **OD value < -10% from the cutoff value: Negative**
When **OD value ≥ -10% from the cutoff value: Repeat test necessary**

When a **repeat test** is required according to the criteria above, use the sample stored in procedure 1. (2) 4), using duplicate wells on the microplate. (The repeat test is preferably conducted by another independent laboratory technician.).

The measurement system is validated by seeing that the absorptions of the negative and positive controls satisfy the criteria below:

(1) **Absorption of all 4 negative control wells < 0.150**
(2) **Absorption of both positive control wells ≥ 1.000**

The following criteria are used to interpret results of the **repeat test**:

(1) **Positive** when **either one of the two wells has an OD value at or above the cutoff value**, or is **within -10% from the cutoff**.
(2) **Negative** when the **OD values of both wells are less than -10% from the cutoff**.

(Separate Attachment 1-2)

Procedures for Dynabott Enfer BSE Test

1. Kit Components

(1) Dynabott Enfer BSE Test components

Reagent Pack (Store at 2-8°C)

Reagent	Content	Storage Conditions	Method of Preparation	Post-Preparation Storage Conditions and Expiry Date
Reagent 3	20 mL × 1 unit	2 - 30°C	Not applicable	Not applicable
Washing Agent 1	100 g powder × 1 bottle	2 - 30°C	Add 50 g of Washing Agent 1 per 1 L of purified water and dissolve	6 months at 2-8°C
Goat Serum	150 µL × 1 unit	2 - 8°C	See section on Anti-PrP Antibody	Not applicable
Conjugate	Concentrated conjugate 100 µL × 1 unit	2 - 8°C	Use Wash Solution 2 to dilute Conjugate according to the dilution ratio specified per lot	Use within 2 hours of preparation
Substrate A	10 mL × 1 bottle	2 - 8°C	Mix equal amounts of Substrates A and B	Store in the dark and use on day of preparation
Substrate B	10 mL × 1 bottle	2 - 8°C		
Centrifuge Plate	2 plates	2 - 30°C	Not applicable	Not applicable
Assay Plate	1 plate	2 - 30°C	Not applicable	Not applicable
Positive Control Well	8 wells	2 - 8°C	Not applicable	Not applicable
Blank Control	30 mL × 1 unit	2 - 30°C	Not applicable	Not applicable

Antibody Pack (Store at -25 - -15°C)

Reagent	Content	Storage Conditions	Method of Preparation	Post-Preparation Storage Conditions and Expiry Date
Reagent 2	3 mL × 1 unit	-25 - -15°C	Not applicable	Not applicable
Anti-PrP Antibody (Rabbit serum)	Concentrated antibody 50 µL ×1 bottle	-25 - -15°C	Use Wash Solution 2 to dilute the Anti-PrP Antibody 500-fold, and the Goat Serum to the dilution ratio specified per lot	Use on day of preparation

Buffer/ Wash Pack (Store at 10-30°C)

Reagent	Content	Storage Conditions	Method of Preparation	Post-Preparation Storage Conditions and Expiry Date
Reagent 1	1 L × 1 unit	10 - 30°C	Not applicable	Not applicable
Washing Agent 2	10x concentrate 500 mL × 1 bottle	10 - 30°C	Add 100 mL of Washing Agent 2 per 900 mL purified water and mix	2 weeks at 10-30°C 1 month at 2-8°C

(2) Ingredients and contents

Reagent	Ingredient	Content (per 100 mL)
Reagent 1	Methanol	16 mL
	Sodium lauryl sulfate (SDS)	15 g
Reagent 2	Proteinase K	0.2 g
Reagent 3	Guanidine hydrochloride	28.659 g
Washing Agent 1	Sodium chloride	100 g*1
Washing Agent 2	Lauromacrogol	0.5 mL
Anti-PrP Antibody	Rabbit anti-PrP serum	100 mL
Conjugate	Horseradish peroxidase-labeled anti-rabbit immunoglobulin (goat)	100 mL
Goat Serum	Normal goat serum	100 mL
Positive Control Well	Synthetic prion peptide	2.4 ng*2
Substrate A	Substrate A (hydrogen peroxide solution)	100 mL
Substrate B	Substrate B (3-aminophthalhydrazide solution)	100 mL
Blank Control	Methanol	16 mL
	Sodium lauryl sulfate	15 g
Assay Plate	96-well microplate	1 plate*3
Centrifuge Plate	96-well microplate	2 plates*3

*1: Per bottle
*2: Per well
*3: No. of plates

2. Required Instruments and Materials

Materials included in Kit

This Kit includes sufficient reagents to assay 45 samples.

Materials not included in Kit

- High quality deionised, distilled or reverse osmosis water must be used (hereinafter referred to simply as purified water)
- Stomacher Biomaster 80 (Seward Ltd.) Homogeniser*
- Homogeniser bags (with filter) (Interscience)
- 2 Skatron Skanwasher® 300 microplate washers (Skatron Instruments AS)*
- iEMS incubator/shaker (Thermo LabSystems)*
- Luminoskan Ascent chemiluminescence reader (Thermo LabSystems)*
- Microplate centrifuge (2750 G or higher)
- Microplate sealers
- Pipettes
- Apparatus for sample collection
- Containers for dilution of the Anti-PrP Antibody and the Conjugate
- Glass or polypropylene containers for dilution of other reagents
- Negative tissue controls (See section on Preparation of Tissue Controls)

* Specific instrument requirements in this assay

3. Instrument Parameter Settings

The pre-set parameter settings in the recommended instruments are shown below. (No setting of parameters is required on the part of the user)

Washer

- 2 separate washers are required for this assay.
- Settings for Wash Protocols 1 and 2:
  - Air pressure: 0.25 atm
  - Volume/ flow rate, adjustment offset >> σv : 1.00
  - Aspirate position (usually 3.00-4.00 mm)
  - Dispense position: 0.00 mm

Wash Protocol 1* (Use Wash Solution 1) Steps:			Wash Protocol 2 (Use Wash Solution 2) Steps:		
# 1	Aspirate	6 seconds	# 1	Aspirate	4 seconds
# 2	Dispense	300 μL	# 2	Wash	3 seconds
# 3	Soak	5 seconds	# 3	Soak	5 seconds
# 4	Aspirate	4 seconds	# 4	Aspirate	2 seconds
# 5	Wash	5 seconds	# 5	Wash	3 seconds
# 6	Soak	5 seconds	# 6	Soak	5 seconds
# 7	Aspirate	3 seconds	# 7	Aspirate	2 seconds
# 8	Wash	2.5 seconds	# 8	Wash	3 seconds
# 9	Soak	5 seconds	# 9	Soak	5 seconds
# 10	Aspirate	2 seconds	# 10	Aspirate	2 seconds
# 11	Wash	2 seconds	# 11	Wash	2 seconds
# 12	Soak	5 seconds	# 12	Soak	5 seconds
# 13	Aspirate	5 seconds	# 13	Aspirate	4 seconds
# 14	End Wash		# 14	End Wash	

* Procedures of Wash Protocol 1 must be performed within a biosafety cabinet.

Shaking incubator

- Shake value: 5 (1400 rpm), Temperature: 34°C

Chemiluminometer

- Plate acceleration: 10, Settle delay: 100, Filter: none, Measurement type: single, Integration time: 300, Lag time: 30 seconds, Measurement count: 1, Photomultiplier (PMT) voltage: default voltage, Plate type: 96 well, Scale factor: up to 8 times

4. Preparation of Reagents

Prepared reagents must be allowed to come to the temperature of the room prior to use.

(1) Wash Solution 1

Prepare Wash Solution 1 by adding 1 liter of purified water to 50 g of powder **Washing**

Agent 1 (**Enfer Wash 1**). Shake until dissolved (or subject to a rotating bottle shaker for 10 minutes), and confirm dissolution before use.
(The prepared Wash Solution 1 may be stored for 6 months at 2-8°C.)

(2) Wash Solution 2

Prepare Wash Solution 2 by diluting the **Washing Agent 2** (**Enfer Wash 2**) concentrate 10-fold using purified water.
(The prepared Wash Solution 2 may be stored for 2 weeks at 10-30°C or for 1 month at 2-8°C.)

(3) Anti-PrP Antibody + Goat Serum Solution

Prepare the Anti-PrP Antibody + Goat Serum Solution by diluting the **Anti-PrP Antibody (Anti-PrP- 1° Ab (Rabbit))** and **Goat Serum (Normal goat Serum (Goat))** using Wash Solution 2 and mixing by inversion. Dilute according to the directions on the labels, as dilution ratios differ per lot.
(The Anti-PrP Antibody + Goat Serum Solution must be used on day of preparation.)

(4) Conjugate Solution

Prepare the Conjugate Solution by diluting the **Conjugate (Enzyme-conjugate- 2° Ab (goat anti-rabbit))** using Wash Solution 2 and mixing by inversion. Dilute according to the directions on the label, as dilution ratio differs per lot.
(The prepared Conjugate Solution must be stored in the dark and used within 2 hours of preparation.)

(5) Substrate Solution

Add an equal volume of **Substrate A** (**Substrate Solution A**) to **Substrate B** (**Substrate Solution B**).
The Substrate Solution must be prepared at least 1 hour before use to allow it to come to the temperature of the room.
(The prepared Substrate Solution must be stored in the dark and used on the day of preparation.)

5. Sample Preparation

1) Prepare a homogenate using **500 ± 40 mg** of the collected bovine medulla oblongata (sample).

2) Place the sample in front of the filter in the homogeniser bag (the bag is compartmented by an internal filter), and confirm that the sample is pushed to the bottom of the bag. Squash the sample between thumb and forefinger to aid subsequent homogenization.

3) Add 7.5 mL of **Reagent 1** (**Enfer Buffer 1 (Bovine)**) into the far side of the filter in the homogeniser bag. Although no particular limit is specified regarding the time interval to homogenization after adding **Reagent 1**, care should be taken to proceed smoothly to the immunoassay.

4) Set the speed setting of the Stomacher homogeniser to 'high', and homogenize the sample for 2 minutes. As the emulsion is prepared using a homogeniser bag with filter, unnecessary matter such as membranes are removed.

Note: After preparation, the homogenised samples must be started immediately on the immunoassay procedure.
The emulsion remaining from the assay is to be stored in the homogeniser bag at room temperature until the results of the first test run are known. Refrigeration must be avoided as it causes crystallization.

6. Immunoassay Procedure

1) The positions A1 and A2 on the **Centrifuge Plate** (**Centrifuge Plate**) are to be reserved for the **Positive Control Wells** (**Peptide Indicator Wells**).
Dispense 180 µL of the **Blank Control** (**Blank Control Reagent (Bovine)**) into each of 4 wells starting at B1, and dispense 180 µL of sample into each of 2 wells per sample.

2) Cover the centrifuge plate with a plate sealer.

3) Centrifuge the plate for 5 minutes at 2750 G.

4) Pipette 20 µL of **Reagent 2** (**Enfer Buffer 2**) directly to the bottom of all wells to be used on the **Assay Plate** (**Enfer Test Plate**).

5) Remove the plate sealer from the centrifuged plate. Collect 100 µL each from the supernatant of the samples and Blank Controls, and transfer to the Assay Plate containing **Reagent 2**.

6) Cover the Assay Plate with a plate sealer.

7) Shake the Assay Plate for 60 minutes at 34°C.

8) Remove the plate sealer and wash the Assay Plate using Wash Solution 1 and <Wash Protocol 1>.

9) Invert the Assay Plate on soft paper and tap well to remove any remaining liquid.

10) Add 150 µL of **Reagent 3** (**Enfer Buffer 3**) to all the wells.

11) Cover the Assay Plate with a plate sealer.

12) Shake the Assay Plate for 15 minutes at 34°C.

13) Remove the plate sealer and wash the Assay Plate using Wash Solution 2 and <Wash Protocol 2>.

14) Invert the Assay Plate on soft paper and tap well to remove any remaining liquid.

15) Remove the wells in positions A1 and A2 from the Assay Plate and replace them with the **Positive Control Wells**.

16) Dispense 150 μL of the prepared Anti-PrP Antibody + Goat Serum Solution into each well.

17) Cover the Assay Plate with a plate sealer.

18) Shake the Assay Plate for 40 minutes at 34°C.

19) Remove the plate sealer and wash the Assay Plate using Wash Solution 2 and <Wash Protocol 2>.

20) Invert the Assay Plate on soft paper and tap well to remove any remaining liquid.

21) Dispense the prepared Conjugate Solution by 150 μL volumes into the Assay Plate.

22) Cover the Assay Plate with a plate sealer.

23) Shake the Assay Plate for 30 minutes at 34°C.

24) Remove the plate sealer and wash the Assay Plate using Wash Solution 2 and <Wash Protocol 2>.

25) Invert the Assay Plate on soft paper and tap well to remove any remaining liquid.

26) Dispense the prepared Substrate Solution by 150 μL volumes into the Assay Plate.

27) Cover the Assay Plate with a plate sealer.

28) Shake the Assay Plate for 10 minutes at 34°C.

29) Remove the plate sealer and use the chemiluminometer to read the luminescence.

A Example of an Assay Plate (Enfer Test Plate) Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	P	P	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
B	B	B	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
C	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
D	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
E	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
F	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
G	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
H	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37	S45	S45

P: Positive Control Well (Peptide Indicator Well)
B: Blank Control (Blank Control Reagent (Bovine))
S1-S45: Samples

Work Flow of Assay

Step	Operation	Time/Temp.	Instrument/ Equipment	Preparation for Subsequent Operations	Precautions
Sample Collection/ Sample Preparation Process					
Collect sample/ Weigh	Sample weight: 500±40 mg		Balance		
Homogenise	Add 7.5 mL of Reagent 1 per 500±40 mg of tissue	2 min. (Speed "High")	Stomacher 80 Homogeniser		Place tissue sample at bottom of filter bag. Following homogenization, let stand at room temperature for a few minutes to allow bubbles to subside.
Transfer homogenate	Transfer homogenate into Centrifuge Plate: 180 µL		Pipette	Likewise transfer 180 µL of the Blank Control into plate	Cover plate with sealer.
Centrifuge	Centrifuge: 2750 G	5 min., Room temp.	Centrifuge	Confirm that incubator temperature is 34°C	Check balance before centrifuging. DO NOT centrifuge at 2-8°C.
Add Reagent 2 (PK)	Add to Assay Plate: 20 µL		Pipette (8-channel)		Add to corner at bottom of well. (Visually confirm addition afterwards)
Dispense sample into plate	Dispense centrifuged supernatant into plate: 100 µL		Pipette (8-channel)		Remove supernatant, watching out for any precipitate.
Incubation 1	Incubate	60 min. at 34°C	LabSystems iEMS Incubator		
Wash 1	Wash using Washing Agent 1 and Protocol 1		Skanwasher 300 microplate washer		After washing, invert and tap several times on paper towel to remove any remaining liquid.
Add Reagent 3	Add 150 µL of Reagent 3		Pipette (8-channel)		

Incubation 2	Incubate	15 min. at 34°C	LabSystems iEMS Incubator	Prepare primary antibody solution (dilute Anti-PrP Antibody+ Goat Serum with prepared Washing Agent 2) Prepare Conjugate (secondary antibody) Solution (dilute Conjugate with prepared Washing Agent 2) Prepare Substrate Solution	
Wash 2	Wash using Washing Agent 2 and Protocol 2		Skanwasher 300 microplate washer	After washing, remove the wells in positions A1 and A2 and set the Positive Control Wells in these positions	After washing, invert and tap several times on paper towel to remove any remaining liquid.
ELISA Process					
Primary antibody	Add primary antibody: 150 µL		Pipette (8-channel)		Confirm that Positive Control Wells are in positions A1 and A2 before adding primary antibody.
Incubation 3	Incubate	40 min. at 34°C	LabSystems iEMS Incubator		
Wash 3	Wash using Washing Agent 2 and Protocol 2		Skanwasher 300 microplate washer		After washing, invert and tap several times on paper towel to remove any remaining liquid.
Conjugate	Add Conjugate: 150 µL		Pipette (8-channel)		
Incubation 4	Incubate	30 min. at 34°C	LabSystems iEMS Incubator		
Wash 4	Wash using Washing Agent 2 and Protocol 2		Skanwasher 300 microplate washer		After washing, invert and tap several times on paper towel to remove any remaining liquid.
Substrate	Add Substrate: 150 µL		Pipette (8-channel)		
Incubation 5	Incubate	10 min. at 34°C	LabSystems iEMS Incubator		
Measure	Measure chemiluminescence		Chemiluminometer		

7. Interpretation

(1) Validation of Test Performance

The control results must be validated before the sample results can be interpreted. Determine the mean luminescence values of the Blank Control and Positive Control Wells. If the following criteria are not met, the assay is invalid, thus a repeat test must be conducted starting from the process of collecting the bovine medulla oblongata (sample) in Section 5. Sample Preparation. Results in the repeat test are evaluated using duplicate wells as well.

1) Blank Control

The median value of the 4 wells of Blank Control must be below 4.0 LU. The median is determined as the mean of the middle 2 values, excluding the maximum and minimum values, for the 4 wells.

2) Positive Control Well

The mean of the Positive Control Wells after subtracting the median of the Blank Control must be confirmed to be within the control range of the lot of Positive Control Wells used (indicated on the Positive Control Well label).

The individual measurements for the Positive Control Wells must not be outside of the mean value of all Positive Control Wells ±30%.

(2) Interpretation of Results

The cutoff value for this Kit is 5.5 LU. The measurements for all samples are interpreted after subtracting the mean of the Blank Control.

If the measurements of both the duplicate wells are 5.5 LU or lower, the sample is interpreted as being negative. Meanwhile, if at least one of the two wells assayed produces a value exceeding 5.5 LU, a complete repeat test using duplicate wells must be conducted for confirmation, starting from the process of collecting the bovine medulla oblongata (sample) in Section 5. Sample Preparation.

In the repeat test results, if at least one of the two wells measured exceeds 5.5 LU, the interpretation of this Kit is positive. As the sample is potentially positive, it requires confirmatory testing. If the result of the repeat test is 5.5 LU or lower for both wells measured, this Kit interprets the sample as negative.

Assay Result of Sample (n=2)

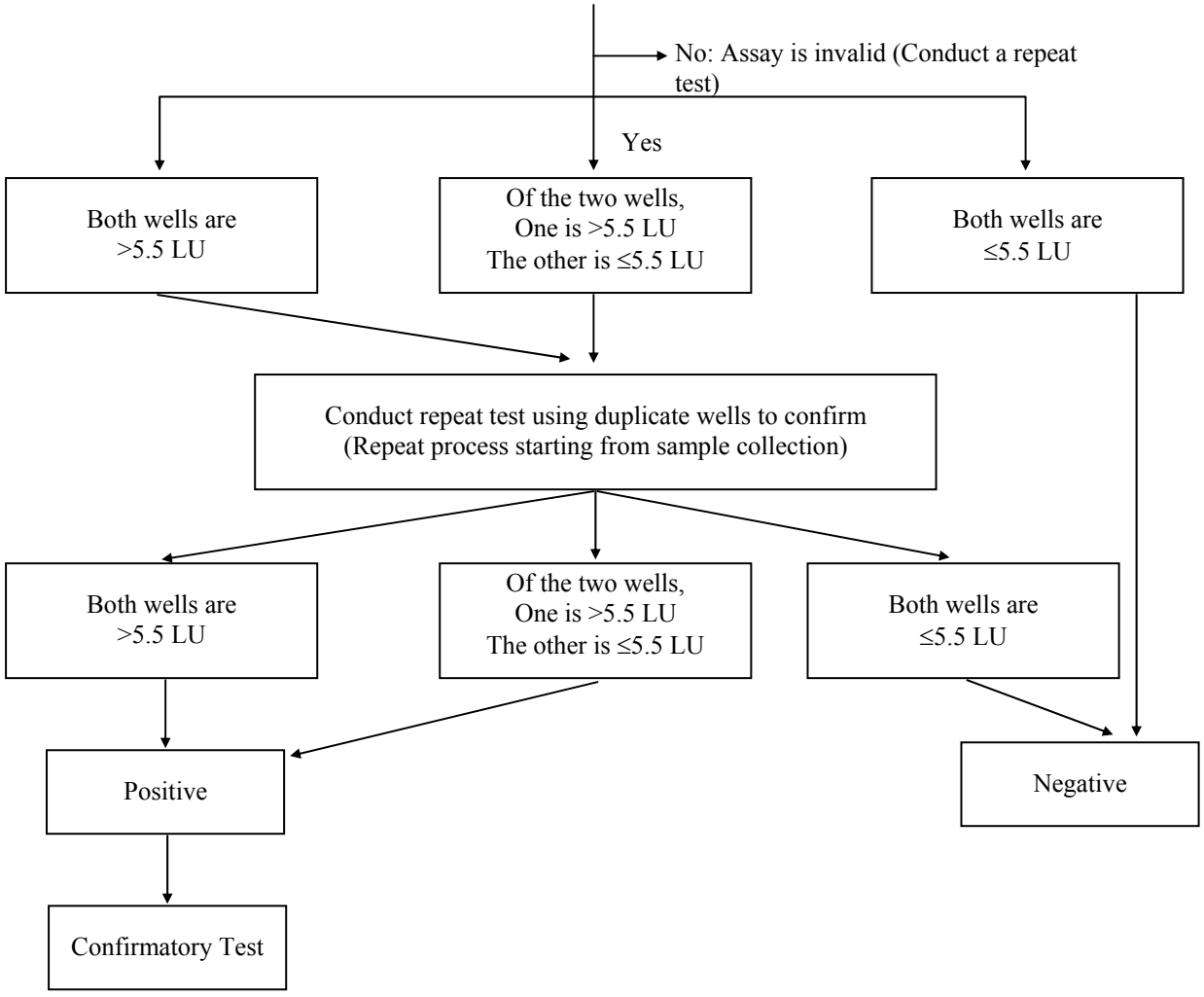
↓

Blank Control

- The median value of the quadruplicate Blank Control measurements must be below 4.0 LU. The median is determined as the mean of the 2 middle values, excluding the maximum and minimum values, for the quadruplicate wells.

Positive Control Well
(Applicable only when the Positive Control Wells provided in this Kit are used.)

- The mean of the Positive Control Wells after subtracting the median of the Blank Control must be confirmed to be within the control range of the lot of Positive Control Wells used.
- The individual measurements for the Positive Control Wells must not be outside of the mean value of all Positive Control Wells ±30%.



8. Partial Use of the Kit

This Kit may be used in portions over four separate runs to test small numbers of samples. The minimum number of samples required for a run in this case is 1.

9. Handling of Samples Interpreted as Positive in this Assay

If the results of the assay make it necessary to conduct a repeat test (if both or either one of the wells are positive), the emulsion which had been stored at room temperature in the homogeniser bag awaiting these results is to be transferred to a 15 mL plastic centrifuge tube for cultivation and stored frozen.

If the result of the repeat test is positive, the emulsion used in the repeat test is also transferred to a 15 mL plastic centrifuge tube for cultivation and frozen, for use in the confirmatory test together with the frozen emulsion from the first test.

If transportation is required for conduct of the confirmatory test, the lid of the 15 mL plastic centrifuge tube for cultivation containing the emulsion is fixed using Parafilm. As a sample for Western Blot, the tube is dispatched wrapped in tissue paper, etc.-- to absorb contents in case the plastic tube breaks or the cap comes loose and as a buffer against shock -- and further packaged in a biohazard can, etc.

Meanwhile if both wells are negative in the repeat test results, the emulsion stored frozen following the first test is disposed of.

(Separate Attachment 1-3)

Procedures for Frelisa® BSE

1. Kit Components

(1) Components of Frelisa® BSE

Frelisa® BSE is a BSE screening reagent kit consisting of 3 reagent sets (Extraction Reagent Set A, Extraction Reagent Set B, and Detection Reagent Set), comprising a total of 17 reagents as shown below.

	No.	Component Reagent	Form	Content per Unit	No. of Units
Extraction Reagent Set A	1	DNase I Solution	Frozen	0.3 mL	1
(Store at -30- -10°C)	2	Collagenase Solution	Frozen	1.8 mL	1
	3	Proteinase K Solution	Frozen	1 mL	2
	4	PK Reaction Stopper	Frozen	1.5 mL	1
Extraction Reagent Set B	5	Homogenizing Solution	Liquid	45 mL	2
(Store at 2-10°C)	6	Surfactant Solution	Liquid	35 mL	1
	7	Concentrating Solution	Liquid	12 mL	1
	8	Solubilizer	Liquid	7 mL	1
	9	Sample Diluent	Liquid	25 mL	1
Detection Reagent Set	10	Antibody-Binding Plate	Well	96 wells	1
(Store at 2-10°C)	11	Enzyme-Labeled Antibody	Liquid	0.6 mL	1
	12	Labeled Antibody Diluent	Liquid	6 mL	1
	13	Negative Control	Liquid	1.5 mL	2
	14	Positive Control	Liquid	1.5 mL	1
	15	Substrate Solution	Liquid	12 mL	1
	16	Wash Solution	Liquid	50 mL	2
	17	Reaction Stopper	Liquid	12 mL	1

(2) Preparation of reagents

The reagents included in the kit must be allowed to return to room temperature before use, and are prepared according to the table below. The detail procedures for preparing Reagents 1, 2 and 3 are given below.

No.	Component Reagent	Form	Method of Preparation
1	DNase I Solution	Frozen	Dilute to 126 times of Surfactant Solution (Prepared Reagent 1)
2	Collagenase Solution	Frozen	Dilute to 21 times against Surfactant Solution (Prepared Reagent 1)
3	Proteinase K Solution	Frozen	Dilute 6-fold using Surfactant Solution (Prepared Reagent 2)
4	PK Reaction Stopper	Frozen	Dilute 31-fold using Concentrating Solution (Prepared Reagent 3)
5	Homogenizing Solution	Liquid	Use as is
6	Surfactant Solution	Liquid	Use as is
7	Concentrating Solution	Liquid	Use as is
8	Solubilizer	Liquid	Use as is
9	Sample Diluent	Liquid	Use as is
10	Antibody-Binding Plate	Well	Use as is
11	Enzyme-Labeled Antibody	Liquid	Dilute 11-fold using Labeled Antibody Diluent
12	Labeled Antibody Diluent	Liquid	Use as is
13	Negative Control	Liquid	Use as is
14	Positive Control	Liquid	Use as is
15	Substrate Solution	Liquid	Use as is
16	Wash Solution	Liquid	Dilute 20-fold using purified water
17	Reaction Stopper	Liquid	Use as is

Prepared Reagent 1: Prepare by diluting the DNase I Solution to 126 times and the Collagenase Solution to 21 times of the Surfactant Solution. A guideline of the volumes to be used per the number of samples to be assayed is given in the table below.

No. of Samples	DNase I Solution (μL)	Collagenase Solution (μL)	Surfactant Solution (mL)
5	12	75	1.5
10	24	150	3.0
20	48	300	6.0
40	84	525	10.5
60	108	675	13.5
80	144	900	18.0
100	180	1125	22.5

Prepared Reagent 2: Prepare by diluting the Proteinase K Solution 6-fold using the Surfactant Solution. A guideline of the volumes to be used per the number of samples to be assayed is given in the table below.

No. of Samples	Proteinase K Solution (μL)	Surfactant Solution (mL)
5	200	1.0
10	300	1.5
20	500	2.5
40	900	4.5
60	1200	6.0
80	1600	8.0
100	2000	10.0

Prepared Reagent 3: Prepare by diluting the PK Reaction Stopper 31-fold using the Concentrating Solution. A guideline of the volumes to be used per the number of samples to be assayed is given in the table below.

No. of Samples	PK Reaction Stopper (μL)	Concentrating Solution (mL)
5	40	1.2
10	60	1.8
20	100	3.0
40	170	5.1
60	250	7.5
80	320	9.6
100	400	12.0

2. Required Instruments and Reagents

(1) Instruments

Electronic balance:	Master Balance LA120S (Sartorius K.K.) or equivalent (reading limit: 0.1 mg, maximum: 10 g or higher, with hood)
Homogenizer:	FastPrep (Qbiogene, Inc.) or Multi-Beads Shocker (Yasui Kikai Corporation)
Constant temperature bath (1):	Constant temperature bath adjustable to 37°C, or a dry block heater
Constant temperature bath (2):	Constant temperature bath adjustable to 100°C, or a dry block heater
Centrifuge:	High-Speed Micro Centrifuge himac CF15R (Hitachi High-Technologies Corporation) or equivalent (main unit capable of 15000 G, with rotor)
Incubator:	Incubator adjustable to 37 °C or plate incubator for ELISA
Microplate washer:	PW-40 (Bio-Rad Fujirebio Inc.) or equivalent
Microplate reader:	Microplate reader Model 550 (Bio-Rad Laboratories, Inc.) or equivalent (with adjustable main/reference wavelengths)
Micropipettes:	200 μL, 1000 μL, 5000 μL, etc.

(2) Consumables

Sample collection apparatus:	Sample collection set (Fujirebio Inc.) or sampling syringe
Homogenizing tube:	2 mL freezing tube (Assist Co., Ltd.)* or grinding tube (Yasui Kikai Corporation)
Metal cone or ceramic beads:	Magnetic metal cone (Yasui Kikai Corporation) or YTZ Balls (Nikkato Corporation)*
Sample tube:	2 mL sampling tube or 2 mL freezing tube (Assist Co., Ltd.)
Micropipette tip:	Various types

* A 2 mL freezing tube (Assist Co., Ltd.) containing 0.5 g of YTZ Balls (Nikkato Corporation) is planned to be released from Fujirebio Inc. as the “Homogenate Tube FR” (tentative).

(3) Instrument Parameters

Microplate washer: The procedures for setting the parameters of PW40 are shown below.

Press IN and OUT simultaneously in the SELECT: RUN screen
↓
PRG: ADD YES
↓
ADD: KIT YES
↓
NAME: Enter a suitable name
↓
Enter, referring to MAIN PARAMETERS and METHOD 1
↓
END OF KIT: NO YES
↓
MET.INTER: OMN OS
↓
Enter, referring to METHOD 2
↓
END OF KIT: YES YES
↓
Nr OF KITS: 1 YES

End

METHOD 1	METHOD 2	MAIN PARAMETERS
MODE: STRIP	MODE: STRIP	PLATE: Flat 01
CROSW ASP.:NO	CROSW ASP.:NO	MANIFOLD: 8
ASP.TIME: 0.1 S	ASP.TIME: 0.1 S	STRIP: -
VOLUME: 800 μL	VOLUME: 800 μL	Nr OF KITS: 1
OVERFLOW: 2.5 mm	OVERFLOW: 2.5 mm	
LIQUID: WASH R1 (W1)	LIQUID: WASH R1 (W1)	
FLOW: 0	FLOW: 0	
Nr OF CYCLES: 1	BOT.ASP.NUMBER: 1	
SOAKING: 0.0 S	Nr OF CYCLES: 4	
MET. INTER: 0MN 0 S	SOAKING: 0.0 S	

Microplate reader: Set the parameters as follows

Blank: Air blank
Main wavelength: 450 nm
Reference wavelength: 600-630 nm

3. Assay Method

(1) Preparation of emulsion

Either a metal cone or ceramic beads may be used as the means for grinding, to be decided by the user.

(1)-1 Add 800 μL of the Homogenizing Solution to the homogenizing tube containing the ceramic beads. (If using a metal cone, first collect the obex of the bovine medulla oblongata into the homogenizing tube, then insert the metal cone and finally add the Homogenizing Solution.)

(1)-2 Collect 200 ± 20 mg of the obex of the bovine medulla oblongata and transfer it into the homogenizing tube.

(1)-3 Seal the lid of the homogenizing tube and set it in the homogenizer.
Recommended homogenizing parameters:
[For ceramic beads]
FastPrep: Speed 6.5, time 45 seconds
Multi-beads Shocker: Speed 3000 rpm, time 1 minute
[For metal cone]
FastPrep: Speed 4.0, time 45 seconds
Multi-beads Shocker: Speed 2000 rpm, time 1 minute

(1)-4 The resulting emulsion is the 20 w/v% emulsion (if lumps are clearly visible, repeat agitation process).

(2) Extraction procedure

(2)-1 Make the Prepared Reagent 1 in the amount required according to the number of samples to be assayed.

(2)-2 Transfer 250 μL of the 20 w/v% emulsion into a 2 mL sample tube and add 200 μL of Prepared Reagent 1 and mix, and incubate for 30 minutes at 37°C.

(2)-3 Make the Prepared Reagent 2 while incubating the above.

(2)-4 When the reaction above is complete, immediately add 100 μL of Prepared Reagent 2 and mix, and incubate for 30 minutes at 37°C.

(2)-5 Make the Prepared Reagent 3 while incubating the above.

(2)-6 When the reaction above is complete, add 100 μL of Prepared Reagent 3 and mix.

(2)-7 Using a high-speed cooling centrifuge, centrifuge at 15000 G for 10 minutes (25-30°C).

(2)-8 After centrifuging, thoroughly remove the supernatant (following decantation, use a micropipette to extract the solution remaining at the bottom of the tube, or let stand inverted for 5 minutes).

(2)-9 Add 50 μL of the Solubilizer to the precipitate obtained from centrifugation, and subject to heat treatment at 100°C for 5 minutes. (Do not mix as it may cause the precipitate to stick to the sides of the tube and not be fully immersed in the Solubilizer.)

(2)-10 Following heat treatment, mix thoroughly to suspend.

(2)-11 After cooling, add 100 μL of the Sample Diluent and mix, to obtain the treated sample. (Sonicate if necessary.)

(3) Detection (ELISA) procedure

(3)-1 Dilute the Enzyme-Labeled Antibody 11-fold using the Enzyme-Labeled Antibody Diluent, according to the number of samples.

(3)-2 Prepare the Wash Solution according to the number of samples (make 500 mL (25 mL Wash Solution + 475 mL distilled water) for up to 45 samples).

(3)-3 Dispense the treated sample (1 well), Negative Control (2 wells) and Positive Control (1 well) by 100 μL each into the wells of the Antibody-Binding Plate. After dispensing, immediately add 50 μL of the diluted Enzyme-Labeled Antibody Solution into each well. Cover the plate with a plate sealer, gently mix, and allow reacting at 37°C for 1 hour.

(3)-4 When the reaction is complete, remove the plate sealer and wash using the diluted Wash Solution (800 μL × 5 times). After washing, invert the plate on paper towel, etc. and tap lightly to remove any remaining Wash Solution in the wells. (For the washing process, the mode must be set individually according to the model of automatic washer used.)

(3)-5 Add 100 μL of Substrate Solution to each well and gently mix, shield from light, and allow to react for 30 minutes at room temperature (20-30°C).

(3)-6 Add 100 μL of the Reaction Stopper to each well and gently mix.

(3)-7 Using a microplate reader, take measurements at main wavelength 450 nm and reference wavelength 600-630 nm.

Example of Assay Plate Layout

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

S1-S93: Sample
NC: Negative Control
PC: Positive Control

4. Interpretation

(1) Cutoff value determination

The cutoff value is defined as the mean of the 2 Negative Control wells (assayed together) plus 0.150.

Cutoff Value = Mean of Negative Controls + 0.150

(2) Validation of the assay system

The mean of the Negative Control absorptions is 0.1 or lower

The absorption of the Positive Control is 1.0 or higher

Sound execution of the assay is confirmed if the reactions of the Positive and Negative Controls satisfy the criteria above. Once the execution of the assay is deemed sound, the results are interpreted according to (3) Interpretation of results below. However, if the criteria above are not met, there may have been a problem in the operations, thus a repeat test must be conducted.

(3) Interpretation of results

If the absorption of the assayed sample is at or above the cutoff value, the sample is interpreted as positive, while if it is less than the cutoff value, the sample is interpreted as negative.

However, samples interpreted as positive using this kit must be subjected to other tests such as immunological, histopathological, or immunohistochemical examinations for confirmation before a final verdict is reached.

(4) Handling of positive cases

A repeat test shall be conducted on samples interpreted as positive in the first test or those showing an absorption only slightly lower than the cutoff (within -10%). The repeat test shall be started from (2) Extraction procedure, using duplicate wells for each sample. If at least one of the wells shows absorption at or above the cutoff in the repeat test, the sample is interpreted as positive.

5. Precautions

- Temperature control must be implemented carefully. If a reaction temperature of 37°C is not maintained during the extraction procedure, enzymatic treatment may not be fully carried out, leading to false negative or false positive results.
- Final mixing or sonication of the emulsion must be thoroughly performed. If large amounts of precipitate are remaining in the treated sample, the washing process in the detection procedure may become insufficient, possibly leading to occurrence of nonspecific reactions.
- Light-shielding must be thorough, as insufficient light-shielding during the enzymatic reaction of the detection (ELISA) procedures may cause the background to rise.

Work Flow

Procedure	Operation	Temp./ Time	Instrument/ Equipment	Preparation for Subsequent Procedures	Precautions
Preparation of Emulsion					
Sample collection			Sampling apparatus		Operations from sample collection to weighing must be performed within safety cabinet
Weighing	200±20 mg		Balance		
Homogenization		Speed: 6.5 Time: 45 sec.	Homogenate tube FR	Make Prepared Reagent 1	If using ceramic beads and FastPrep
Extraction Procedure					
Sampling	Collect emulsion: 250 µL		Micropipette		Operations from sampling to dilution must be performed within safety cabinet
Enzymatic treatment-1	Add Prepared Reagent 1: 200 µL	37°C, 30 min.	Repeating dispenser/ Water bath/ Dry block heater	Make Prepared Reagent 2	
Enzymatic treatment-2	Add Prepared Reagent 2: 100 µL	37°C, 30 min.	Repeating dispenser/ Water bath/ Dry block heater	Make Prepared Reagent 3	
Concentration	Add Prepared Reagent 3: 100 µL / Centrifuge	15000 G, 10 min (25-30°C)	Repeating dispenser/ High-speed centrifuge		Pool and sterilize waste solution before disposing
Solubilization	Add Solubilizer: 50 µL	100°C, 5 min.	Repeating dispenser/ Water bath/ Dry block heater		
Dilution	Add Sample Diluent: 100 µL				
Detection (ELISA) Procedure					
Preparation of Labeled Antibody	Dilute Enzyme-Labeled Antibody: × 11				
Preparation of Wash Solution	Dilute Wash Solution: × 20				
Primary reaction	Sample: 100 µL NC: 100 µL × 2 PC: 100 µL + Labeled Antibody Solution: 50 µL	37°C, 1 hour	Micropipette/ Repeating dispenser/ Incubator		Carefully prevent droplets from entering nearby wells when dispensing the Labeled Antibody
Washing	0.8 mL × 5 times		Microplate washer		Sterilize waste solution before disposing
Enzymatic reaction	Add Substrate Solution: 100 µL	Room temp., 30 min.	Repeating dispenser/ Incubator		Carefully light shield
Reaction stop	Add Reaction Stopper: 100 µL		Repeating dispenser		Remove bubbles before measuring
Measurement of absorption			Microplate reader		Discard used plates as contaminated items

6. Precautions on Handling and Usage

[General]

1) The kit contents must be used strictly according to the specified doses and methods of use.
2) Do not use on samples other than bovine medulla oblongata.
3) Samples interpreted as positive using this kit must be subject to other tests such as immunological (Western Blot), histopathological, or immunohistochemical examinations for confirmation.
4) Diagnosis of bovine spongiform encephalopathy (BSE) shall be conducted according to the scheme established by the Japanese government.

[To the User]

1) All procedures for extracting prion protein from bovine medulla oblongata shall be performed within a safety cabinet as a general rule, and care must be taken in handling to avoid generating droplets and aerosols.
2) The operator shall wear rubber gloves or protective gloves, mask, protective eyeglasses, and other protective clothing when operating.

[Usage]

1) A sample stored at 2-10°C must be used within 24 hours. For longer periods of time, the sample must be stored frozen.
2) Disposable products shall be used where possible for work clothes and instruments.
3) Care must be taken to avoid cross-contamination between samples during sample extraction and detection procedures.
4) The Prepared Reagents prepared using DNase I Solution, Collagenase Solution and Proteinase K Solution must be used within 4 hours.
5) Reagents of differing lot numbers are not to be mixed for use.
6) Extraction Reagent Set A must be mixed after thawing. Extraction Reagent Set B and the Detection Reagent Set must be returned to room temperature (20-30°C) prior to use.
7) Use a fresh sample tip for collection of each sample.
8) The Antibody-Binding Plate must be washed the specified number of times, and confirmed to be thoroughly clean.
9) Dispense the substrate without delay upon completing washing of the Antibody-Binding Plate.
10) After dispensing the Substrate, shield from light during the reaction.
11) Measurements must be taken within 10 minutes after dispensing the Reaction Stopper.
12) The Wash Solution may show a crystalline precipitate when stored at 4°C, in which case the precipitate must be dissolved at 37°C before use.
13) As the Concentrating Solution consists of 2-butanol, a hazardous substance, it should not be used near fire.

[Handling]

1) Perform all testing in a clean environment. When reusing reagents, take great care to avoid contamination by bacteria.
2) Do not use expired kits.
3) Do not use components that are abnormal in appearance or content.
4) Do not freeze/thaw the Extraction Reagent Set A for 12 cycles or more.
5) Remove only the required number of strips. Return unnecessary strips to the bag with the desiccant and seal and store at 2-10°C.
6) Only the required amounts of reagents are taken out and brought to room temperature prior to use. Any reagents removed but not to be used must be returned swiftly to the storage temperature for storage.
7) If any assay materials are spilled, wipe clean with hypochlorous acid solution (2% effective concentration) and soak in the same for 120 minutes or longer.
8) Contaminated materials such as samples and used instruments must be sterilized according to one of the methods below before being disposed of.
   - Autoclave sterilization (134-138°C, 3 atm, 20 minutes or longer)
   - Soak in 3% SDS solution at 100°C for 5 minutes or longer
   - Soak in 3% SDS solution and autoclave (120°C, 10 minutes or longer)
   - Soak in hypochlorous acid solution (2% effective concentration) for 120 minutes or longer.
   - Saturate in 1 mol/L sodium hydroxide solution for 60 minutes or longer.

[Storage]

1) Store out of reach of children.
2) Avoid storage in direct sunlight or a humidified environment, etc. as it will cause deterioration.