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NippIBL BSE Test Kit II

1. Kit components

(1) Components of **NippIBL® BSE Test Kit II Pretreatment Reagents**

No.	Components	contents (1 box)
1,	Reagent A (Homogenization buffer)	100 mL x 1
2,	Reagent B (Proteinase K)	1.0 mL x 1
3,	Reagent C (Microbial serine protease)	1.0 mL x 1

(2) Components of **NippIBL® BSE Test Kit II Detection Reagents**

No.	Components	contents (1 box)
4,	Antibody-coated plate (96 wells)	1
5,	Enzyme-labeled antibody (concentrated)	0.4mLx1
6,	Dilution buffer for Enzyme-labeled antibody	12mLx1
7,	Positive control (lyophilized)	1
8,	Negative control	3mLx1
9,	TMB substrate solution	15mLx1

Accessory item: Plate cover seal

(Storage condition): 2-8°C

* Instruments and materials not included in the kit:

- Sterilizing solution; 2% (active chlorine concentration) hypochlorous acid solution or 1 mol/L sodium hydroxide solution
- Paper towel
- Protective glass and mask
- Disposable glove
- Refrigerator
- Incubator (37°C for plate incubation)
- Incubator (56°C for digestion reaction)
- Incubator (100°C for enzyme inactivation)

- Centrifuge for 2mL tube
- Tissue homogenizer (for example, FastPrep (MP BIOMEDICALS) or Multi Beads Shocker (Yasui Instrument))
- Micro plate reader (for reading at 450nm and 580-650nm)
- Plate washer
- Micro pipette (20-1000μL) and disposable tip
- Test tube (5, 10 or 20 mL)
- Graduated cylinder (100mL) and beaker (2L)
- Distilled water
- Electric balance

- **Nippl ELISA reagents and Pretreatment Equipment set**
  - Biomasher® (I: crushing rod, II: filter tube) (Figure 1)
  - Recovery tube (with zirconia balls) (Figure 1)
  - Reaction stop solution (0.5 M H_2SO_4)
  - Concentrated wash buffer

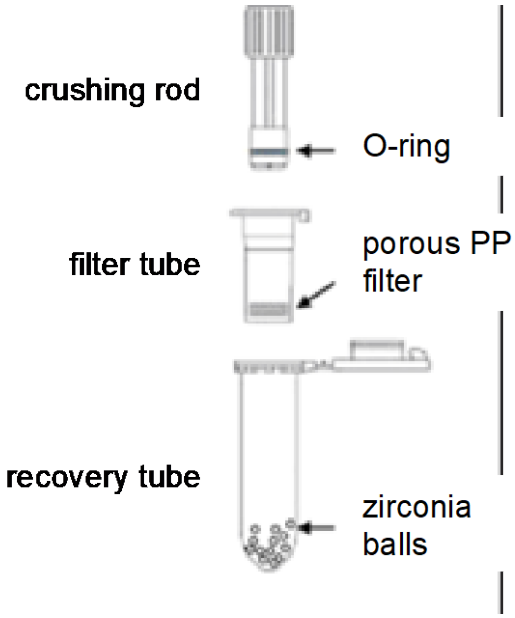

Figure 1 Biomasher® and Recovery tube

2. Preparation of reagents and set up of instruments. (for sample preparation)

1) Set the two incubators at 56°C and 100 °C respectively.

2) Preparation of the enzyme mix solution

Gently agitate Reagent A, B and C. Mix reagents A, B and C to a 100:1:1 ratio. A guideline of the volumes per number of samples to be used is shown in the following table.

Number of samples	Reagent A（mL）	Reagent B（μL）	Reagent C（μL）
2	3 mL	30 μL	30 μL
10	11 mL	110 μL	110 μL
15	16 mL	160 μL	160 μL
20	21 mL	210 μL	210 μL
25	26 mL	260 μL	260 μL
30	31 mL	310 μL	310 μL
35	36 mL	360 μL	360 μL
40	41 mL	410 μL	410 μL
45	46 mL	460 μL	460 μL
50	51 mL	510 μL	510 μL

The enzyme mix solution should be prepared freshly before every test, and kept at 4-8°C before use.

3. Sample preparation procedure

1) Collect 110±20mg of the bovine brain stem tissue at the obex.

2) Set the BioMasher filter tube inside the collection tube, insert the collected tissue, and push the Biomasher crushing rod in securely until the sample contacts the filter surface.

3) Centrifuge the recovery tube with the BioMasher in step 2 at 15,000 x g for 30 sec.

4) Remove and discard the BioMasher filter tube and BioMasher crushing rod. Add one (1) mL of the enzyme mixture to the tube for recovery.

5) Tighten the lid of the recovery tube and agitate with a tissue homogenizer. When using the FastPrep (MP BIOMEDICALS), agitate at a speed setting of 4.0 m/s for 30 seconds. When using the Multi Bead Shocker (Yasui Instrument), agitate at 2000 rpm for 30 seconds.

6) Incubate the recovery tube at 56℃ for 10 min.

7) After gentle agitation, incubate the recovery tube at 100℃ for 10 min.
8) Cool the recovery tube to room temperature.
9) After cooling, agitate the recovery tube gently. The sample is then ready for detection.

4. Preparation of reagents (for detection procedure)

1) Wash buffer; Dilute 25mL of concentrated wash buffer with 975mL of distilled water. The Wash buffer can be stored at 4-8°C for at most 2 weeks after dilution.
2) Enzyme-labeled antibody solution; Dilute the Enzyme-Labeled Antibody (concentrated) solution 30 fold with the Enzyme-labeled antibody Dilution Buffer. Prepare 100 µL of diluted enzyme-labeled antibody solution per one sample.
3) TMB substrate solution; Transfer the required amount of substrate solution into a test tube before use, and do not return any excess back into the bottle. Prepare 100 µL of substrate solution per one sample.
4) Positive control; Reconstitute the Lyophilized positive control with 2mL of distilled water and agitated gently. After reconstitution, Positive control can be stored at 4-8°C.

5. Detection (ELISA) procedure

1) Dispense 100 uL of the negative control (2 wells) into the antibody-coated plate.
2) Dispense 100 uL of the sample (1 well per 1 sample) and positive control (1 well) into the antibody-coated plate.
3) Cover the plate with the sealing film and incubated at 37°C for 1 hr.
4) After removing the sealing film, wash the plate with wash buffer. For plate washing, use more than 300µL of wash buffer per well. Repeat the washing step 4 times. After washing, the washing solution should be removed completely from the well. Do not leave the plate in this condition for 5 minutes or longer.
5) Dispense 100 µL of the Enzyme-labeled antibody solution into each well.
6) Cover the plate with the sealing film and incubate at 4-8°C for 30 minutes.

7) After removing the sealing film, wash the plate with wash buffer. For plate washing, use more than 300μL of wash buffer per well. Repeat the washing step 6 times. After washing, remove the washing solution completely from the well. Do not leave the plate in this condition for 5 minutes or longer.
8) Dispense 100 μL of the TMB substrate solution into each well. Shield the plate from light (for example, covering the plate with aluminum foil) and incubate at room temperature for 30 minutes. The positive control well should turn blue gradually.
9) Dispense 100 μL of the Reaction stop solution into each well.
10) Make sure that the bottom of the plate is clean. Within 30 minutes after adding the reaction stop solution, measure the OD using a micro plate reader, at the main wavelength of 450 nm and a reference wavelength of 580-650 nm (any reference wavelength within this range will yield consistent results).

6. Interpretation

1) Validation of the measurement system (ELISA)

Validate the measurement system by confirming that the OD values at 450 nm for both the negative and positive controls are satisfied with the criteria shown below.

[Average OD of 2 negative control wells] ≦ 0.20

[OD of positive control] – [Average OD of negative control wells] ≧ 1.20

2) Cut off value

[Cut off value] = [Average OD of negative control wells] + 0.30

3) Interpretation of results

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

Positive: OD of sample ≧ cutoff value

Negative: OD of sample < cutoff value

4) Remarks of interpretation

1. If the measurement system (ELISA) fails, repeat the procedure from step 5 using samples that have been prepared in step 3.
2. When the OD value of the assayed sample is at or above the cutoff

value, or slightly lower than -10% from the cutoff value, the test should be repeated.

- Retests should be started from sample collection (step 3).
- Two wells per one sample should be used for the retest.

3. If the OD value of retested sample, in at least one of the wells, is at or above the cutoff value, the sample is interpreted as positive.
4. If the sample is interpreted as positive, other confirmation tests should be conducted, for instance, western blotting, histopathological or immuno-histochemical examination etc

NippIBL® BSE Test KitIIWork flow