

aj ROBOSCREEN

Complete Solutions for BIQ-Quantification

BetaPrion[®] SCRAPIE EIA-Test^{*}

Test for *in vitro* purification and detection of sheep PrP^{res}

Manual

*) for research use only

BetaPrion[®] SCRAPIE EIA-Test

Cat.No. 0104000103

Abbreviations:

HRP (Horseradish peroxidase), PrP (Prion protein) BSA (Bovine serum albumin), TMB (Tetramethylbenzidine); ELISA (Enzyme-linked immunosorbent assay), RT (Room Temperature), PrP^{res} (PrP resistant to proteinase K, PrP^{sen} (PrP sensitive to proteinase K)

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1. Introduction

The BetaPrion[®] SCRAPIE EIA-Test consists of two modules, the BetaPrion[®] Purification Kit, which includes the purification tools and the BetaPrion[®] Detection Kit, which is based on a sensitive ELISA.

The BetaPrion[®] SCRAPIE EIA-Test Kit is a continuous 100 min test for the detection of PrP^{res} in sheep brain samples. Specimens of sheep brain are homogenized and incubated with Proteinase K. Solubilized PrP^{res} is captured by a specific monoclonal anti-PrP antibody coated to the wells of a microtitre strip. Bound PrP^{res} is detected with a HRP-conjugated anti-PrP antibody. The wells are washed and a substrate solution is added. The developed colour indicates the existence of PrP^{res} in the specimen in comparison to a negative and a positive control in case of overshoot the declared cut-off.

The BetaPrion[®] SCRAPIE EIA-Test contains sufficient reagents to analyze 90 sheep brain samples

2. Components not provided with the kit

For the Purification kit

- Homogenisation device, Ribolyzer, FastPrep or FastPrep-24 System (MP Biomedicals formerly Q-BIOgene, USA), Precellys24 (Bertin, France)
- BSE sample syringes
- 15 or 50 ml tubes
- 2 ml tubes
- Single channel pipetting tools (adjustable, 100-1000 µl, 10-100 µl) and tips
- Desktop centrifuge
- Thermostate mixer
- Syringe, e.g. JG25-1,5NC 1ml
- Needles (blunt end), 25Gx1.5"
- Vortex mixer
- Refrigerator 4-8 °C

For the Detection Kit

- Single channel pipetting tools (adjustable, 100-1000 µl, 10-100 µl) and tips
- 15 ml tubes
- Multichannel pipette (adjustable, 30-300 µl)
- Microplate reader
- Microplate washer

3. BetaPrion® SCRAPIE Purification Kit

3.1. Sampling

Sheep brain of the obex region or Cerebellum must be used as starting material for the BetaPrion® SCRAPIE EIA-Test.

Two opportunities for sampling:

- (1) Cut 350 ± 50 mg nervous tissue from this area from the left **or** the right side of the brainstem with a scalpel and weigh the sample to ensure the correct amount.
- (2) Sample preparation using the AJ Roboscreen BSE sample syringe.

3.2. Kit components

Short name	Type/content	Quantity
Homogenisation tubes	Transparent tubes (2 ml) with red cap containing Homogenisation buffer (1.3 ml) and ceramic beads, ready to use. Store at RT	96 tubes
P1	Proteinase K buffer in transparent bottle with red label. Store at RT	1 x bottle (24 ml)
P2	Proteinase K solution in transparent tube with yellow cap. Store at 4-8 °C	1 x tube (0.6 ml)
P3	Precipitation solution in brown bottle with red label containing detergents, ready to use. Store at RT	1 x bottle (45 ml)
P4	Solubilisation buffer in transparent bottle with red label containing detergent solution, ready to use. Store at RT	1 x bottle (4 ml)

3.3. Preparation of the Reagents

Ready to use reagents:

The homogenisation tubes and the solubilisation buffer (P4) are ready to use reagents. The precipitation solution (P3) is ready to use after storing at 4-8 °C for at least 1 h.

Reconstituted reagents:

Proteinase K buffer (P1) is required for the dilution of Proteinase K (P2) and must be used as follows ($5 \pm 0.5 \mu\text{l}$ Proteinase K in $200 \pm 20 \mu\text{l}$ Proteinase K buffer):

Number of the samples	Proteinase K buffer (P1)	Proteinase K (P2)
8	2 ml	50 μ l
40	10 ml	250 μ l
60	15 ml	375 μ l
90	20 ml	500 μ l

3.4. Storage conditions and shelf life of the components

Store the homogenisation tubes, the Proteinase K buffer, and the solubilisation buffer at room temperature (15-25 °C). The precipitation solution can be stored at RT or 4-8 °C, respectively. The tube containing Proteinase K has to be stored at 4-8 °C. The guaranteed shelf life of the reagents is 12 months.

The reconstituted Proteinase K solution is stable up to 3 hours at room temperature (15-25 °C) or up to 8 hours at 4-8 °C.

3.5 Test Procedure

1. Pre-cool the RT stored precipitation solution at 4-8 °C for at least 1 h.
2. Place the brain sample (350 \pm 50 mg) into a homogenisation tube and homogenize for 45 sec at maximum speed (6.5) using one of the approved homogenizers. Homogenized brain samples can be stored at -20 ± 2 °C for up to 12 months.
3. Transfer 200 \pm 20 μ l of homogenized sample with a 1-ml syringe using a fine needle (blunt end needle 25Gx1.5") to a new 2 ml Eppendorf tube.
4. Add 200 \pm 20 μ l of the reconstituted Proteinase K solution, mix by vortexing (2 sec at maximum speed) or inverting the tubes 6 times.
5. Incubate at 37 \pm 1 °C for 15 \pm 1.5 min with continuous shaking (thermomixer, 750 rpm) .
6. Add 400 \pm 40 μ l pre-cooled precipitation solution, mix by vortexing (2 sec at maximal speed) or inverting the tubes 6 times (check the solution for complete mixing) and incubate the sample for 15 \pm 1.5 min at RT.
7. Centrifugate at 16.000 \pm 1.600 g for 5 \pm 0.5 min. Discard the supernatant carefully and removed the remaining supernatant by tipping the tubes on a paper towel.
8. Add 25 \pm 2.5 μ l solubilisation buffer to the pellet and heat at 99 \pm 1 °C for 5 \pm 0.5 min with continuous shaking (thermomixer, 750-1400 rpm). Cool down the tubes for at least 5 min at RT. Samples can be stored up to 6 h at 4-8 °C.
9. Analyze the sample for PrP^{res} protein using the BetaPrion[®] SCRAPIE Detection Kit.

4. BetaPrion[®] SCRAPIE *Detection Kit*

4.1. Kit components

Short name	Type/content	Quantity
D1	Immunostrips coated with monoclonal anti-PrP capture antibody, stabilized, ready-to-use.	1 immunoplate (12 x 8 wells)
D2	Washing buffer (10x concentrate) in a transparent bottle, containing 0.01 % Na-Merthiolat as preservative.	1 x bottle (100 ml)
D3	Positive control in transparent tubes (2 ml) with blue caps containing lyophilized recombinant bovine prion protein.	2 x tubes
D4	Negative control in transparent tubes with white caps, ready-to-use.	3 x tubes (3 x 0.1 ml)
D5	Horseradish peroxidase conjugate in amber bottle with black cap containing 8x concentrate of conjugated anti-PrP antibody. Preservative: 0.1 % Proclin 300.	1 x bottle (2.5 ml)
D6	Dilution buffer in transparent bottle containing buffered saline solution with BSA, ready to use. Preservative: 0.1 % Proclin 300.	1 x bottle (20 ml)
D7	Tetramethylbenzidine solution in amber bottle with red cap containing a liquid stabilized TMB solution.	1 x bottle (1 ml)
D8	Peroxide substrate solution in amber bottle with green cap containing peroxide solution.	1 x bottle (1 ml)
D9	Staining buffer in transparent bottle containing buffered saline solution.	1 x bottle (20 ml)
D10	Stop solution in transparent containing 1 M sulphuric acid, ready-to-use.	1 x bottle (25 ml)
	Sealing tape	1 x tape

4.2. Preparation of the reagents

Allow immunostrips, washing buffer, dilution buffer, staining buffer and stop solution to equilibrate to room temperature prior performing the assay.

Ready to use reagents:

After prewarming to room temperature of the immunostrips (D1), open the bag with a scissors and remove the needed strips. Unused strips should be stored at 4-8 °C up to 1 week. Dilution buffer (D6) and Stop solution (D10) are ready to use reagents.

Reconstituted reagents:

Washing solution (D2):

Dilute the 10 x concentrated wash buffer with distilled water (add 900 ml of aqua dest to the 100 ml concentrate) before the washing step of the immunoassay.

Positive control (D3):

Add 0.5 ml of diluted HRP conjugate to positive control tube and vortex for 2 sec at the beginning of the immunoassay.

Negative control (D4) :

Add 0.5 ml of diluted HRP conjugate to negative control tube and vortex for 2 sec at the beginning of the immunoassay.

HRP conjugate (D5) :

Dilute the 8x HRP conjugate with dilution buffer (for 1 plate add 14 ml dilution buffer to 2 ml of conjugate) for diluted HRP conjugate.

Staining solution (D7 + D8 + D9)

Mix 12 ml of staining buffer (D9) with 300 µl of TMB solution (D7) and 180 µl peroxide substrate solution (D8) during the washing step of the immunoassay.

4.3. Storage conditions and shelf life of the components

Reconstituted reagents have a shelf life as follows:

Short name	Reagent	Shelf life
D1	Coated immunostrips after opening of the bag.	1 week at 4-8°C
D2	1X washing solution.	1 week at RT
D5	Diluted HRP conjugate	4 h at 4-8°C
D8	Mixed staining solution.	2 h at RT in dark vials

Store all reagents of the Detection kit at 4-8°C. The guaranteed shelf life of the reagents is 12 months.

4.4. Immunoassay

Assay procedure for using 1 immunoplate (12x8 strips):

If you use only individual strips please recalculate the reagent volumes required accordingly. To dispense the controls and samples into the wells of the immunoplate please follow the immunoplate partitioning shown in the pipetting scheme (chapter 7)

1. Add 125 ± 12.5 µl of diluted HRP conjugate to the solubilized sample resulted from the BetaPrion[®] Purification Kit step 9 and mix it by at least 3 x inverting of the tubes or vortexing 2 sec.
2. Pipet 4x 100 ± 10 µl of one negative and 2x 100 ± 10 µl of one positive control, respectively.

3. Pipet $100 \pm 10 \mu\text{l}$ of the diluted sample per well of the coated immunostrips.
4. Cover the strips with sealing tape and incubate for $45 \pm 4.5 \text{ min}$ at RT.
5. Remove the sealing tape and wash the strips 5 times with $200 \pm 20 \mu\text{l}$ diluted wash buffer either manually or by using the Columbus washer, program BSE-5.
6. Prepare the staining solution.
7. Dispense $100 \pm 10 \mu\text{l}$ of prepared staining solution per well.
8. Incubate for $10 \pm 1 \text{ min}$ in the dark at room temperature (for this step do not seal the plate with the sealing tape).
9. Terminate reaction by adding $150 \pm 15 \mu\text{l}$ of stop solution per well.
10. Read the O.D. at 450 nm and 620 nm as reference wave length using the microplate reader within 15 minutes after termination of the reaction.

5. Interpretation of the results

The cut-off value is defined as 0.2 optical density $\text{OD}_{450/620 \text{ nm}}$ and must be used for the discrimination of SCRAPIE positive samples from negative samples. The interpretation of data is done as follows:

- All samples with an $\text{OD}_{450/620 \text{ nm}}$ below 0.2 are classified SCRAPIE negative.
- Samples with an $\text{OD}_{450/620 \text{ nm}} \geq 0.2$ are classified initially reactive and must be re-tested in duplicate using the original homogenate. If one of the two duplicate readings has an $\text{OD}_{450/620 \text{ nm}} \geq 0.2$ the sample is classified SCRAPIE positive and must be handled according to the respective national guidelines.

Validation of the test

The $\text{OD}_{450/620 \text{ nm}}$ value of the positive control must be at least 1.0 and of the $\text{OD}_{450/620 \text{ nm}}$ value of the negative control must be below 0.1. The plate must be repeated, when two of the negative controls have an $\text{OD}_{450/620 \text{ nm}} \geq 0.1$.

6. Precautions

Prions are a unique class of pathogens which exhibits unusual resistance to conventional chemical and physical decontamination methods. A Biosafety level 3 laboratory is necessary for testing of TSE material. National legal regulations have to be considered. The quality of results generated with BetaPrion[®] SCRAPIE EIA-Test depends on compliance with the good Laboratory Practices (GLP).

Use disposable consumable supplies whenever possible. For decontamination of TSE positive material soak instruments in 2.5% sodium hypochlorite or 1 M NaOH for at least 1 hour or clean contaminated surfaces with those solutions. Liquids and waste should be decontaminated by autoclaving at 134°C for 1 hour.

7. Appendices

Pipetting scheme for the coated Microstrips

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

Distribution of the controls and samples:

N = Negative control

P = Positive control

1-90 = Samples

How to contact **aj** ROBOSCREEN

If you are using the Internet access our web-site at www.aj-roboscreen.com.

Please contact us by:

- E-mail at: info@aj-roboscreen.com
- Phone at: +49-341-9897340
- or Fax at: +49-341-989734199.

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8. Warranty notice/Additional general remarks

During the warranty period the BetaPrion® SCRAPIE EIA-Test allows precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.

Safety Statements

- **Homogenisation buffer** must not be classified according to the Directive 1999/45/EC.
- **Buffer P1** contains Sodium Dodecylsulfate, which is harmful (Xn) according to the Directive 1999/45/EC. Risk (R) and Safety Statements (S) are listed below:
 - S26 = in case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
 - R22 = harmful if swallowed.
 - R36/37/38 = irritating to eyes, respiratory system and skin.
 - S36 = wear suitable protective clothing.
- **Buffer P2** contains proteinase K, which is harmful (Xn) according to the Directive 1999/45/EC. Risk (R) and Safety Statements (S) are listed below:
 - R42 = may cause sensitization by inhalation.
 - R43 = may cause sensitization by skin contact.
- **Buffer P3** must not be classified according to the Directive 1999/45/EC.
- **Buffer P4** must not be classified according to the Directive 1999/45/EC.
- **Buffer D1** must not be classified according to the Directive 1999/45/EC.
- **Buffer D2** contains Tris[hydroxymethyl]aminomethan, which is irritant (Xi) according to the Directive 1999/45/EC. Risk (R) and Safety Statements (S) are listed below:
 - R36/38 = irritating to eyes and skin.
- **Buffer D3** must not be classified according to the Directive 1999/45/EC.
- **Buffer D4** must not be classified according to the Directive 1999/45/EC.
- **Buffer D5** must not be classified according to the Directive 1999/45/EG.
- **Buffer D6** must not be classified according to the Directive 1999/45/EC.
- **Buffer D7** contains Tetramethylbenzidine and organic solution, which are toxic and harmful (T, Xn) according to the Directive 1999/45/EC. Risk (R) and Safety Statements (S) are listed below:
 - S1 = keep locked up.
 - S45 = in case of accident or if you feel unwell, seek medical advice immediately.
 - R25 = toxic if swallowed.
 - R20/21 = harmful by inhalation and contact with skin
 - R36 = irritating to eyes
 - S26 = in case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
 - S28 = after contact with skin, wash immediately with plenty of water.
 - S36 = wear suitable protective clothing.

- **Buffer D8** contains peroxide, which is corrosive (C) according to the Directive 1999/45/EC. Risk (R) and Safety Statements (S) are listed below:
 - R34 = causes burn
 - S3 = keep in a cool place
 - S26 = in case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
 - S36/37/39 = wear suitable protective clothing, gloves and eye/face protection.
 - S45 = in case of accident or if you feel unwell, seek medical advice immediately.

- **Buffer D9** must not be classified according to the Directive 1999/45/EC.

- **Buffer D10** contains Sulphuric acid, which is corrosive (C) according to the Directive 1999/45/EC. Risk (R) and Safety Statements (S) are listed below:
 - R35 = causes severe burns
 - S2 = keep out of the reach of children
 - S26 = in case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
 - S30 = never add water to this product.
 - S45 = in case of accident or if you feel unwell, seek medical advice immediately.

9. General comments

Homogenized brain samples in homogenisation buffer can be stored at -20°C for up to 12 months. For further use, thaw the samples at RT and vortex for 2 sec at maximal speed. Samples can be submitted to a maximum of 3 freezing-thawing cycles.

All contents of the BetaPrion[®] SCRAPIE EIA-Testt are produced under the guidelines of quality accordingly to the DIN EN ISO 9001:2000 requirements.

aj ROBOSCREEN

**Delitzscher Straße 135, Laborgebäude
D-04129 Leipzig (Germany)**

Phone +49-341-9897340
 Fax +49-341-989734199
 Email info@aj-roboscreen.com
 Internet www.aj-roboscreen.com