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Complete Solutions for BIQ-Quantification

# BetaPrion<sup>®</sup> HUMAN EIA Test Kit

Test for detection of human prion protein\*

*Manual*

\*) for research use only

BetaPrion<sup>®</sup> HUMAN EIA Test Kit  
Cat. no.: 0104000104

*For research use only*

The BetaPrion<sup>®</sup> HUMAN EIA Test Kit can be used in combination with BetaPrion<sup>®</sup> BSE Purification Kit (Cat.No.: 0104100102) for sample preparation.

**Abbreviations:**

HRP (Horseradish peroxidase), TMB (Tetramethylbenzidine); ELISA (Enzyme-linked immunosorbent assay), RT (Room Temperature)

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## 1. Introduction

The BetaPrion<sup>®</sup> HUMAN EIA Test Kit bases on a sensitive sandwich ELISA that uses two specific anti-human prion protein antibodies.

Samples and standard are mixed with dilution buffer for the first ELISA incubation step. After washing the diluted HRP conjugate reacts onto the plate with the bound antigen. The evidence of bound HRP-antibody-antigen complexes is shown by TMB/H<sub>2</sub>O<sub>2</sub> staining.

For quantification of human PrP samples lyophilized standards are provided with the kit that contain defined amounts of recombinant human prion protein.

## 2. Kit components

Short name	Type/content	Quantity
<b>Antibody coated plate</b>	Immunostrips coated with anti-human prion protein capture antibody, stabilized, ready-to-use.	12 x 8 wells
<b>10X washing buffer</b>	Washing buffer (10X concentrate) in a transparent bottle, containing 0.01 % Na-Merthiolat as preservative.	1 x bottle (100 ml)
<b>Positive control</b>	Positive controls in transparent tubes (2 ml) with blue caps containing positive control lyophilized.	3 tubes
<b>Negative control</b>	Negative controls in transparent tubes (2 ml) with white caps containing negative control lyophilized.	3 tubes
<b>Standards rhu-PrP</b>	Standards in transparent tubes containing 6.25 ng (clear cap), 3.125 ng (violet cap) and 1.56 ng (yellow cap) of recombinant human prion protein.	3 x 3 tubes
<b>10X HRP-conjugate</b>	10X concentrate of Horseradish peroxidase conjugate of anti-human prion protein detection antibody in amber bottle with black cap. Preservative: 0.1 % Proclin 300.	1 x bottle (1.5 ml)
<b>Dilution buffer</b>	Dilution buffer in transparent bottle containing buffered saline solution with BSA, ready to use. Preservative: 0.1 % Proclin 300.	1 x bottle (25 ml)
<b>TMB solution</b>	Tetramethylbenzidine solution in amber bottle with red cap containing a liquid stabilized TMB solution.	1 x bottle (1 ml)
<b>Peroxide solution</b>	Peroxide substrate solution in amber bottle with green cap containing peroxide solution.	1 x bottle (1 ml)
<b>Staining buffer</b>	Staining buffer in transparent bottle containing buffered saline solution.	1 x bottle (20 ml)
<b>Stop solution</b>	Stop solution in transparent bottle containing 1 M sulphuric acid, ready-to-use.	1 x bottle (25 ml)
	Sealing tape	2 x tape

### 3. Preparation of the components

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, 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 and Stop solution are ready to use reagents.

#### Reconstitution of reagents:

##### *Washing solution:*

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.

##### *Staining solution*

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

##### *HRP conjugate :*

Dilute the 10X HRP conjugate with dilution once concentrated for samples, controls and standards (e.g. 9 ml dilution buffer to 1 ml conjugate).

##### *Positive controls, negative controls and standards :*

Add 1 ml of dilution buffer to 1 tube and vortex it for 2 s. Concentration of controls and standards are calculated for 1 ml volume.

### 4. Storage conditions and shelf life of the components

Store all reagents of the Detection kit at 4-8 °C. The guaranteed shelf life of the reagents is 6 months.

Reconstituted reagents have a shelf life as follows:

Short name	Reagent	Shelf life
<b>strips</b>	Coated immunostrips after opening of the bag.	1 week at 4-8 °C
<b>washing buffer</b>	1X washing solution.	1 week at RT
<b>conjugate</b>	Diluted HRP conjugate	8 h at 4-8 °C
<b>staining solution</b>	Mixed staining solution.	2 h at RT in dark vials

## 5. Immunoassay

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

If you use only individual strips please recalculate the reagent volumes required accordingly.

### Assay procedure

1. Pipet 100  $\mu$ l of the diluted sample or 100  $\mu$ l of controls or standards per well onto the ELISA plate.
2. Cover the strips with sealing tape and incubate for  $60 \pm 6$  min at  $37^\circ\text{C}$ .
3. Remove the sealing tape and wash the strips 3 times with  $200 \pm 20$   $\mu$ l diluted wash buffer either manually or by using a washer.
4. Prepare the HRP conjugate solution.
5. Pipet 100  $\mu$ l of the HRP conjugate solution to each well.
6. Cover the strips with sealing tape and incubate for  $60 \pm 6$  min at RT.
2. Remove the sealing tape and wash the strips 5 times with  $200 \pm 20$   $\mu$ l diluted wash buffer either manually or by using a washer.
1. Prepare the staining solution.
2. Dispense  $100 \pm 10$   $\mu$ l of prepared staining solution per well.
3. Incubate for  $15 \pm 1$  min in the dark at room temperature (for this step do not seal the plate with the sealing tape).
4. Terminate reaction by adding  $150 \pm 15$   $\mu$ l of stop solution per well.

## 6. Measurement and interpretation of the results

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.

The OD<sub>450/620 nm</sub> value of the positive control should be  $> 2.5$  and value of negative control should be  $< 0.2$ .

Use the standard values to generate a curve related to the concentration of human PrP standard. Calculate the concentration of human prion protein in the sample depending on the dilution of the sample used in the test.

The calculation for the human prion protein in the sample should be done inside the linear range of the standard curve between 0.5 and 2.0 OD<sub>450/620 nm</sub>.

## 7. Appendices

### How to contact **aj** ROBOSCREEN

If you are using the Internet access our web-site at [www.aj-roboscreen.com](http://www.aj-roboscreen.com).

Please contact us by:

- E-mail at: [info@aj-roboscreen.com](mailto:info@aj-roboscreen.com)
- Phone at: +49-341-9897340
- or Fax at: +49-341-989734199.

## 8. Warranty notice/Additional general remarks

During the warranty period the BetaPrion<sup>®</sup> HUMAN EIA Test Kit 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

- **Coated plates** must not be classified according to the Directive 1999/45/EC.
- **Washing buffer** 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.
- **Control** must not be classified according to the Directive 1999/45/EC.
- **Dilution buffer** must not be classified according to the Directive 1999/45/EG.
- **HRP conjugate** must not be classified according to the Directive 1999/45/EC.
- **TMB solution** 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.
- **Peroxide solution** 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.
- **Staining buffer** must not be classified according to the Directive 1999/45/EC.
- **Stop solution** 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

All contents of the BetaPrion<sup>®</sup> HUMAN EIA Test Kit are produced under the guidelines of quality accordingly to the DIN EN ISO 9001:2000 requirements. The use of this test is allowed for research and development testing only.

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