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Seprion™-PAD

Microsens Seprion Technology™

Tools for Protein Aggregation Disease Diagnostics

CJD-EIA

96 well CJD EIA Test

Creutzfeldt-Jakob Disease (CJD)
Abnormal Prion Protein Test Kit EIA

User Guide

For research use only

CJD-EIA

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Overview

The Microsens Seprion Technology™ **CJD-EIA** Creutzfeldt-Jakob Disease (CJD) Abnormal Prion Protein Test Kit is an antigen-capture enzyme immunoassay (EIA) for detection of the abnormal conformer of the prion protein (PrP^{CJD}) in brain and lymphoid tissues from individuals affected by Creutzfeldt-Jakob Disease (CJD). It is designed to rapidly identify samples containing disease-associated PrP^{CJD} with minimal sample handling, and can be automated for high throughput applications.

This kit is for research use only.

Description and Principles

The Microsens **CJD-EIA** Test Kit uses a proprietary method (patent pending) that allows detection of abnormal (aggregated) prion protein. A PrP^{CJD}-specific ligand is immobilized on the surface of the CJD antigen-capture plate. Test samples are prepared by homogenizing the tissues and then diluting the sample with working plate diluent. After the sample is applied to the plate, the disease associated conformer binds to the immobilized ligand with high affinity. The plates are washed to remove unbound materials, including the normal conformer of the PrP protein. Following incubation with conditioning buffer, the captured antigen is then detected using a PrP-specific antibody that has been conjugated to horseradish peroxidase (HRPO). The plate is washed to remove unbound conjugate and a peroxidase substrate is added. Colour development is related to the relative amounts of PrP^{CJD} captured by the ligand immobilized in the microtiter plate well.

Interpretation of sample results is based on the sample absorbance. A sample whose signal value is less than the cut-off value is considered to be negative by the Microsens **CJD-EIA** Test Kit. Samples whose signal value is initially greater than or equal to the cut-off and is confirmed in duplicate are classified as positive for PrP^{CJD}. A confirmatory assay such as immunohistochemistry is suggested for all positive test results.

Precautions

• Do not expose the TMB substrate to strong light or oxidizing agents. Use clean or disposable plasticware for dispensing TMB.
• Care should be taken to avoid contamination of kit components. Do not use components past their expiration dates, and do not intermix components from different kit lots.
• Some kit components contain sodium azide as a preservative (see description of kit components). Care should be taken to prevent contaminating the anti-PrP-HRPO conjugate with azide-containing solutions.
• Store all reagents at 2°–7°C. Bring reagents to room temperature (18°–25°C) prior to use, and return to proper storage temperatures after use (see Storage of Prepared Reagents).
• Use separate dispensing trays for each reagent used in the assay. Avoid cross-contamination of the TMB substrate with the diluted conjugate solution. Do not pour unused TMB solution back into the bottle.
• Do not allow microtiter plates to sit more than 5 minutes between wash steps and the addition of reagents.

Safety Information

• The conditioning buffer contains chaotropic agents; avoid contact with skin and mucous membranes.
• The TMB substrate may irritate skin and eyes. Avoid direct contact.
• Plate diluent 1 contains high concentrations of detergents; avoid direct contact.

11. Aspirate the final wash.

12. Add 100 µL of the prepared HRPO-conjugate anti-PrP antibody solution to the microwell plate. Cover the plate and incubate for 60–75 minutes at room temperature (18°–25°C). No shaking required.

13. Aspirate the antibody solution and wash the wells five times with approximately 350 µL of 1X wash solution 2. Aspirate and fill the wells after each wash.

14. Aspirate the final wash.

15. Add 100 µL of the TMB substrate. Cover the plate and incubate for 30 minutes (±1 minute) at room temperature (18°–25°C); protect the plate from direct light. Do not use adhesive film during this step.

16. Quench the HRPO-mediated color development by adding 100 µL of acid stop solution to the plate. The plate can be held for up to 30 minutes in the dark at 18°–25°C prior to reading the optical density.

17. The signal for each sample is determined by reading the absorbance of the microwells at 450 nm using a reference wavelength of 620 nm to 650 nm (depending on available microtiter plate reader filters).

18. In order to assign the test result for each sample please see, 'Assignment of test results' below.

Plate Validity

For the assay to be valid, the negative control mean signal (NCx) must have a value less than 0.120 and the positive control mean signal (PCx) must have a value ≥0.400.

Calculations
Calculation of negative control mean signal (NCx):
NCx = (Signal of microwell A1 + Signal of microwell B1)/2

Calculation of positive control mean signal (PCx):
PCx = (Signal of microwell C1 + Signal of microwell D1)/2

Assignment of test results

The assay cut-off is determined by the negative control mean signal plus 0.12. Any sample that gives a signal below this cut-off is termed 'negative'. Any sample tested once that gives a signal equal to or above this cut-off is termed 'initial reactive'. If subsequently confirmed in duplicate the sample can then be termed 'positive' and we recommend that such samples should be investigated by immunohistochemistry.

Kit Components

Store all components at 2˚–7˚C (36˚–45˚F).

Item (1 x 96 well plate)

A	Antigen-capture plate - 1 plate
N	Negative control - Nonreactive with antigen-capture plate; preserved with sodium azide, 1 x 1 mL
P	Positive control - Noninfectious, reactive with capture plate; preserved with sodium azide, 1 x 1 mL
D1	Plate diluent component 1- preserved with sodium azide, 5 mL
D2	Plate diluent component 2- 1 x 200 µL
R	Reconstitution buffer- 5 mL
CB	Conditioning buffer- preserved with sodium azide, 12 mL
CC	Conjugate concentrate-preserved with Bronidox L and methylisothiazolone, 300 µL
CD	Conjugate diluent- buffer with detergents and protein stabilizers; preserved with kathon, 12 mL
W1	10X wash solution 1- preserved with sodium azide, 120 mL
W2	10X wash solution 2- preserved with gentamicin, 120 mL
T	TMB substrate - 12 mL
S	Stop solution - 12 mL

Materials and Equipment Required (Not Provided)

• Precision pipettes and multi-channel pipettes suitable for delivering between 25 and 200 µL.
Reagent volumes listed in the Test Procedure require pipette precision of ±5%.
• Graduated cylinders for wash solutions
• Hard plastic or adhesive plate covers, and reagent-dispensing trays
• 96-well plate reader (equipped with 450-nm and 620–650-nm filters) and washer
• Homogeniser, such as FastPrep instrument (manufactured by Thermo-Electron) or Precess 48 (manufactured by Bertin)
• Dilution plates and extended-length homogenate transfer pipette tips
• Deionized water
• Plate shaker

Preparation of Reagents

Wash Solutions (Wash Solution 1, Wash Solution 2)
The wash solution concentrates should be brought to room temperature (18°–25°C) and **mixed to ensure dissolution of any precipitated salts.** Each wash concentrate must be diluted 1:10 with distilled or deionized water before use (e.g., 40 mL of concentrate plus 360 mL of water per plate to be assayed).

Plate Diluent Component 2
Plate diluent component 2 (D2) is supplied as lyophilized material. The solution is prepared by adding 200µl of reconstitution buffer, letting the vial stand for approximately one minute and then mixing gently. Use within one hour of preparation.

Working Plate Diluent
Plate diluent component 1 (D1) should be brought to room temperature (18°–25°C). **Important: Invert vial gentley a few times to ensure mixing.** Prepare the working plate diluent by adding 1 part plate diluent component 2 (D2; prepared as above) and 25 parts plate diluent component 1 (D1); mix thoroughly (e.g., 140 µL D2 + 3.5 mL D1 per plate). Approximately 3.5 mL of working plate diluent is required per plate. Working plate diluent should be used within two hours of preparation.

Negative and Positive Controls
Negative and positive controls are supplied lyophilized. Reconstitute each control by adding 1 mL of reconstitution buffer. Allow the solution to stand for approximately one minute and then mix vigorously. Use within two hours of preparation.
DO NOT DILUTE NEGATIVE OR POSITIVE CONTROLS INTO WORKING PLATE DILUENT.

HRPO-Conjugated Anti-PrP Antibody Solution
The HRPO-conjugated anti-PrP antibody solution is prepared by diluting the conjugate concentrate (CC) into the conjugate diluent (CD) as indicated on the label (for example, a 1:100 dilution would require 120 µL conjugate concentrate to 12 mL of conjugate diluent).
IMPORTANT: Refer to the conjugate concentrate (CC) label for correct dilution factor. Diluted conjugate should be prepared and used within four hours. For optimal performance only use conjugate in combination with the Kit Product Code as indicated on the conjugate packaging.

Acid Stop Solution

All reagents should be at room temperature (18°–25°C) before use. Before starting the test, prepare the solutions to be used in the assay. Mix all reagents by gentle swirling. Controls (negative and positive) should be mixed vigorously and tested in duplicate. A plate cover should be used to cover the plate for the duration of the assay.

Storage of Prepared Reagents

Negative/Positive control: Two hours at 18°–25°C or Six months at -20°C
Plate diluent 2: One hour at 18°–25°C or Six months at -20°C
Working plate diluent: Two hours at 18°–25°C
HRPO:anti-PrP solution: Four hours at 18°–25°C
Wash solution 1–1X: One week at 18°–25°C
Wash solution 2–1X: One week at 18°–25°C
Store any unused portion of plates in a dark, desiccated, sealed container.

Test Procedure

Important: Cover each assay plate with a solid plastic or adhesive plate cover during all reagent incubations. If reagent incubations are conducted in a biosafety cabinet, plates must be covered with adhesive sheets.

1. Set up a template indicating where the sample positions are located on the antigen-capture plate and the dilution plate. Reserve wells A1, B1, C1, D1 for the kit controls. Working plate diluent can be added to the dilution plate before or after the sample. The ratio is 30 µL of working plate diluent per 120 µL of sample homogenate.

2. If homogenates have been frozen, ensure that all homogenates are thoroughly defrosted and mixed prior to assay. If possible, resuspend the homogenates by inversion. Carefully pipette the sample by extending a homogenate transfer pipette tip through any homogenisation beads. Carefully dispense each sample into the dilution plate, taking care to avoid creating bubbles in the homogenate or leaving any residual homogenate on the edges of the dilution plate well.

3. After the homogenate is diluted, thoroughly mix samples, taking care to avoid bubbles. Proceed with the assay within two hours.

4. Mix the negative control, and immediately dispense 100 µL into each well A1 and B1 of the antigen capture plate. Do not add plate diluent to the negative control.

5. Mix the positive control, and immediately dispense 100 µL into each well C1 and D1 of the antigen capture plate. Do not add plate diluent to the positive control.

6. Dispense 100 µL of sample mixed with plate diluent into the appropriate wells of the antigen-capture plate.

7. Cover the antigen-capture plate with a plate cover. Incubate the plate for 2.0 hours at room temperature (18°–25°C) on a plate shaker (450 rpm).

8. Aspirate the contents of the wells. Wash the wells six times with approximately 350 µL of 1X wash solution 1. Aspirate and fill the wells after each wash. Aspirate the final wash.

9. Add 100 µL of conditioning buffer to each well, cover the plate and incubate at room temperature (18°–25°C) for 10 minutes (±1 minute). No shaking required

10. Aspirate the conditioning buffer and wash each well three times with approximately 350 µL of 1X wash solution 2. Aspirate and fill the wells after each wash.