

Human Major prion protein,PRNP ELISA Kit

96 Tests

Operating instruction

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

Synonyms

Major prion protein,PRNP,PrP,PrP33-35C,ASCR,PrP27-30, Major prion protein,prion protein,ASCR; AltPrP; CD230; CJD; GSS; KURU; PRIP; PrP; PrP27-30; PrP33-35C; PrPc; p27-30; CD230 antigen; prion-related protein

Search name

Human Major prion protein ELISA KIT ,Human PRNP ELISA KIT ,Human PrP ELISA KIT ,Human PrP33-35C ELISA KIT ,Human ASCR ELISA KIT ,Human PrP27-30 ELISA KIT ,Human Major prion protein ELISA KIT ,Human prion protein ELISA KIT ,Human ASCR ELISA KIT ,Human AltPrP ELISA KIT ,Human CD230 ELISA KIT ,Human CJD ELISA KIT ,Human GSS ELISA KIT ,Human KURU ELISA KIT ,Human PRIP ELISA KIT ,Human PrP ELISA KIT ,Human PrP27-30 ELISA KIT ,Human PrP33-35C ELISA KIT ,Human PrPc ELISA KIT ,Human p27-30 ELISA KIT ,Human CD230 antigen ELISA KIT ,Human prion-related protein ELISA KIT

Intended use

This immunoassay kit allows for the in vitro quantitative determination of human major prion protein ,Human PrP concentrations in serum ,Human Plasma ,Human tissue homogenates and Cell culture supernates and Other biological fluids.

Test principle

The microtiter plate provided in this kit has been pre-coated with an antibody specific to PrP. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for PrP and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain PrP ,Human biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm \pm 2 nm. The concentration of PrP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials and components

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	1 \times 20ml
Assay Diluent A	1 \times 10ml
Assay Diluent B	1 \times 10ml
Detection Reagent A	1 \times 120 μ l
Detection Reagent B	1 \times 120 μ l
Wash Buffer(25 x concentrate)	1 \times 30ml
Substrate	1 \times 10ml
Stop Solution	1 \times 10ml
Plate sealer for 96 wells	5
Instruction	1

Other supplies required

Microplate reader.
Pipettes and pipette tips.
EP tube
Deionized or distilled water.

Storage of the kits

The **Assay Plate ,Human Standard ,Human Detection Reagent A and Detection Reagent B** should be stored at -20 $^{\circ}$ C upon being received. After receiving the kit ,Human **Substrate should be always stored at 4 $^{\circ}$ C**.Other reagents are kept according to the labels on vials. But for long term storage ,Human please keep the whole kit at -20 $^{\circ}$ C. The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown ,Human provided it is stored as prescribed above.

Note: Because of the inherent stability of lyophilized material ,Human Nova lifetech may ship these materials at ambient temperature.

Sample collection and storage

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g at 2°C - 8°C within 30 minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, Human tissue was rinsed with 1X PBS to remove excess blood, Human homogenized in 20 mL of 1X PBS and stored overnight at ≤ -20°C. After two freeze-thaw cycles were performed to break the cell membranes, Human the homogenates were centrifuged for 5 minutes at 5000 × g. Remove the supernate and assay immediately or aliquot and store at ≤ -20°C.

Cell culture supernates and Other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 2-8°C, Human otherwise samples must be stored at -20°C (1 month) or -80°C (2 months) to avoid loss of bioactivity and contamination.
2. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
3. Influenced by the factors including cell viability, Human cell number and also sampling time, Human samples from cell culture supernatant may not be detected by the kit.
4. Sample hemolysis will influence the result, Human so hemolytic specimen can not be detected.
5. When performing the assay slowly bring samples to room temperature.

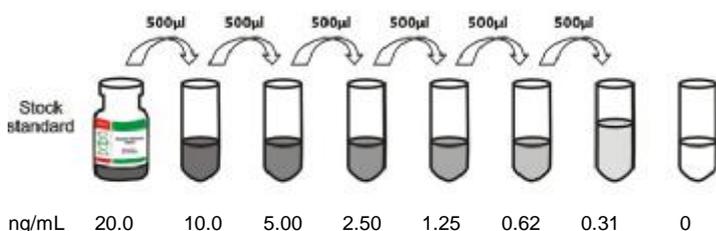
Limitations of the procedure

1. Nova Lifetech is only responsible for the kit itself, Human but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. The kit should not be used beyond the expiration date on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.
4. If samples generate values higher than the highest standard, Human further dilute the samples with the Sample Diluent and repeat the assay. Any variation in standard diluent, Human operator, Human pipetting technique, Human washing technique, Human incubation time or temperature, Human and kit age can cause variation in binding.

Reagent preparation

Wash Buffer - If crystals have formed in the concentrate, Human warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 20.0 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard (20.0 ng/mL). The Sample Diluent serves as the zero standard (0 ng/mL).



Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A and B (1:100), Human respectively.

Assay procedure

Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37°C directly.). **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4°C until the kit's expiry date. Prepare all reagents, Human working standards and samples as directed in the previous sections. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, Human users must determine the optimal sample dilutions for their particular experiments.

1. Add 100 µl of **Standard**, Human Blank, Human or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37°C.
2. Remove the liquid of each well, Human don't wash. Add 100 µl of **Detection Reagent A** working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37°C. Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
3. Aspirate each well and wash, Human repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µl) using a squirt bottle, Human multi-channel pipette, Human manifold dispenser or autowasher. and let it sit for 1-2 minutes. Complete removal of liquid at each step is essential to good performance. After the last wash, Human remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of **Detection Reagent B** working solution to each well. Cover with a new Plate sealer. Incubate for 1 hour at 37°C.

5. Repeat the aspiration/wash process for 5 times as conducted in step 3..
6. Add 90 µl of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate within **15-30 minutes** at 37°C. Protect from light.
7. Add 50 µl of **Stop Solution** to each well. If color change does not appear uniform ,Human gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well at once ,Human using a microplate reader set to 450 nm.

Note:

1. Absorbance is a function of the incubation time. Therefore ,Human prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step ,Human without interruption.
2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction ,Human and avoid foaming and mix gently until the crystals have completely dissolved. **The reconstituted Standards Detection Reagent A and B can be used only once.** This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting ,Human ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting.
3. To ensure accurate results ,Human proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips ,Human DO NOT let the strips DRY at any time during the assay.
4. For each step in the procedure ,Human total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
5. To avoid cross-contamination ,Human change pipette tips between additions of each standard level ,Human between sample additions ,Human and between reagent additions. Also ,Human use separate reservoirs for each reagent.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
7. Duplication of all standards and specimens ,Human although not required ,Human is recommended.
8. Substrate Solution is easily contaminated. Please protect it from light.
9. The web version of manual is only for reference ,Human subject to the instruction shipping with the kit.

Specificity

This assay recognizes recombinant and natural human PrP. No significant cross-reactivity or interference was observed.

Note:

Limited by current skills and knowledge ,Human it is impossible for us to complete the cross- reactivity detection between human PrP and all the analogues ,Human therefore ,Human cross reaction may still exist.

Detection Range

0.31 ng/mL -20.0 ng/mL.

Calculation of results

Average the duplicate readings for each standard ,Human control ,Human and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative ,Human construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the PrP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation ,Human such as curve expert 1.3. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted ,Human the concentration read from the standard curve must be multiplied by the dilution factor.

Important note:

1. Limited by the current condition and scientific technology ,Human we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit
2. The final experimental results will be closely related to validity of the products ,Human operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
3. Kits from different batches may be a little different in detection range ,Human sensitivity and color developing time. Please perform the experiment exactly according to the instruction attached in kit while electronic ones from our website is only for information.
4. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results.
5. Do not remove microtiter plate from the storage bag until needed.
6. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.
7. Use fresh disposable pipette tips for each transfer to avoid contamination.
8. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
9. Even the same operator might get different results in two separate experiments. In order to get better reproducible results ,Human the operation of every step in the assay should be controlled. Furthermore ,Human a preliminary experiment before assay for each batch is recommended.
10. Each kit has been strictly passed Q.C test. However ,Human results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different

batches might arise from above factors ,Human too.

11. Kits from different manufacturers for the same item might produce different results ,Human since we haven't compared our products with other manufacturers.
12. The instruction manual also suit for the kit of 48T ,Human but all reagents of 48T kit is reduced by half.
13. Valid period: six months.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye ,Human hand ,Human face ,Human and clothing protection when using this material.

TYPICAL DATA

This graph data is shown as an example. A standard curve must be generated **each time** the assay is run.

