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User's Manual

Nrp1 (Mouse) ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Nrp1 (Mouse) ELISA kit comprises of one 96-well microplate, some buffers, some diluents, the substrate solution and the stop solution. Mouse neuropilin-1 can be measured quantitatively and specifically. This product is intended for research use only.

Principles of Testing

By using the Nrp1 (Mouse) ELISA kit for target protein, the sandwich ELISA system is build. The capture antibody is coated on a 96-well microplate. Standards or samples are added to the microwells, allowing target protein to bind to the capture antibody in proportion to the concentration of target protein in the samples. After wash unbound target protein, detection antibody is added and attaches to the target protein bound to coated antibody. After washing away all unbound detection antibody, initiate the substrate reaction. After substrate reaction, add the stop solution to the microwell plate. Then the relative amount of target protein bound in each well can be determined from the yellow color using a microplate reader. The concentration of target protein in each sample can be obtained by comparing the optical density (OD) of the sample to the OD of the standards on the plate.

Reagents And Materials Provided

1. Mouse Neuropilin-1 standard: Recombinant mouse Neuropilin-1 (lyophilized powder): 1 vial. Dilution ratio: 1:10 – 1:640.
2. Anti-Mouse Neuropilin-1 capture antibody: Rat anti-mouse Neuropilin-1 monoclonal antibody (50% Glycerol/PBS): 60 µL × 1 vial. Dilution ratio: 1:200.
3. Anti- Mouse Neuropilin-1 detection antibody: Biotin conjugated rat anti-Mouse Neuropilin-1 monoclonal antibody: 110 µL × 1 vial. Dilution ratio: 1:101.
4. Streptavidin conj. peroxidase: Streptavidin conjugated peroxidase (SA-HRP), 110 µL × 1 vial. Dilution ratio: 1:101.
5. Microwell strips: Non coated microwell strips, 8-well × 12 strips.
6. Coating Buffer: Carbonate buffer solution (ready-to-use), 12 mL × 1 bottle.
7. Blocking Agent: Contains BSA and sucrose (ready-to-use), 24 mL × 1 bottle.
8. Sample Diluent: Buffer mostly for diluting samples (ready-to-use). Contains BSA, Tween 20 and HAMA-Blocker, 50 mL × 1 bottle.
9. Wash Concentrate: Buffer for washing microwells (20X) Contains Tween 20, 50 mL × 1 bottle.
10. SA-HRP Diluent: Buffer mostly for diluting SA-HRP (ready-to-use) Contains BSA, 15 mL × 1 bottle.
11. Substrate Solution: TMB/H_2O_2 solution (ready-to-use), 20 mL × 1 bottle.
12. Stop Solution: 0.25 mol/L sulfuric acid (ready-to-use), 20 mL × 1 bottle.

Note:

1. Reconstitute "Standard" by dissolving lyophilized Mouse Neuropilin-1 with distilled water. The volume of distilled water is specific for each lot, and it is indicated in the label on the tube of "Mouse Neuropilin-1

standard. To avoid repeated freezing and thawing, prepare aliquots.

2. Kit components must be brought to room temperature (20-30°C) prior to use.

Storage

The Mouse Neuropilin-1 standard, Anti-Mouse Neuropilin-1 capture antibody, Anti- Mouse Neuropilin-1 detection antibody, Streptavidin conj. peroxidase, must be stored at -20 ~ -30°C, other components stored at 2-8°C. All reagents are stable for 12 months after shipment.

Reagent Preparation

Wash Concentrate

Prepare Wash solution by diluting Wash Concentrate, 1:20 with the deionized water prior to use (ex. Add 50 mL of Wash Concentrate to 950 mL of deionized water). After preparation, it is stable for 2 weeks at 2-8°C.

Standard

For preparing Conditioned standard, standard should be diluted 1:10 with Sample Diluent or culture medium. With the 1:10 dilution as top Conditioned standard, 5 to 7 points should be made with Sample Diluent or culture medium by 2-fold serial dilution. Use the same diluent without any standard added as blank (zero conc.). "Standard" must be stored below -20°C. To avoid repeated freezing and thawing, prepare aliquots.

Capture antibody

The capture antibody should be diluted with Coating Buffer. Dilution must be used immediately and cannot be stored for future use.

Detection antibody

The Biotin conjugated detection antibody should be diluted with Sample Diluent. The HRP conjugated detection antibody should be diluted with SA-HRP Diluent. Dilution must be used immediately and cannot be stored for future use.

Streptavidin conj. peroxidase

In case SA-HRP solution is needed, prepare SA-HRP solution by diluting Streptavidin conj. peroxidase (SA-HRP) with SA-HRP Diluent. Dilution must be used immediately and cannot be stored for future use.

Microplate Coating with capture antibody

STEP 1. (Coating)

1. Dilute capture antibody with Coating Buffer.
2. Soon after mixing by repeated inversion, dispense 100 µL to each microwell with multichannel pipette and let stand overnight at 2-8°C with seal (or other cover to avoid evaporation).

Note: 1) Use a new conical 15 mL tube to mix the antibody solution.

2) Please confirm the optimal dilution ratio with the data sheet of the Neuropilin-1 (Mouse) ELISA kit.

STEP 2. (Washing)

Wash the wells with saline (saline: NaC l: 9.0g / 1,000 mL) 2 times.

Washing Method:

1. Discard the liquid out of the wells. Be careful not to detach strips from the microcup frame.
2. Gently pour wash fluid into the empty wells.
3. Repeat the discarding and refilling steps.
4. Finally, tap the plate several times on paper towel to remove any contents.

Note:1) If an autowasher is used, optimal washing times vary depending on the instrument used and its setting.

2) Wash as quickly as possible, NOT let wells dry up.

STEP 3. (Blocking)

1. Add 200 µL of Blocking Agent to each well and incubate for one hour at room temperature.
2. Dump out the contents of the wells over sink before use.

Note: 1) Discarding of antibody and addition of blocking should be done as soon as possible as to not to let the cup become dry.

2) After completion of the blocking step, the coated plate can be stored for a long period of time when properly dried. After discarding blocking agent, apply fan or other drying method and leave at room temperature for 3 hours to overnight to ensure proper drying. Store the plate at 2-8°C, under strictly controlled moisture conditions. The color development of dried plates may be decreased when compared with plates that were never dried.

Assay Procedure

Duplicate assay is recommended. A standard curve must be run with each assay.

1. Sample incubation

1) Dilute standard 1:10 with Sample Diluent or culture medium. For preparing dilution series, with the 1:10 dilution as top Conditioned standard, 5 to 7 points should be made with Sample Diluent or culture medium by 2-fold serial dilution. Then use buffer solution used for preparation as Blank.

2) Dilute samples with optimal dilution in Sample Diluent or culture medium.

Note: a. When assayed with culture supernatant as sample, culture medium should be used to dilute the standard.

b. Sample Diluent contains HAMA-Blocker to block the effect of human anti- mouse antibodies (HAMA) that may be present in human serum.

3) Add 100 µL of each Conditioned standard points and samples to coat well. Incubate for 1 hour at room temperature (primary reaction).

Note: a. The Antigen-antibody reaction starts on addition. Addition should be completed as quickly as possible. It is recommended that standard and sample are diluted on a separate microplate in advance, and then added to the antibody coated plate with a multichannel pipette.

b. When adding solution to the microplate, avoid touching the inner wall of microcup with the pipet tip. This technique avoids non-specific reaction.

2. Washing

Wash the wells 4 times with Wash solution following the Washing Method.

For Washing Method, refer to "Microplate Coating with capture antibody, STEP 2".

3. Detection antibody incubation

1) Add 100 µL of diluted detection antibody to each well.

2) Incubate for 1 hour at room temperature.

Note: a. Avoid touching the inner wall of the wells with the pipette tip. Otherwise, it could cause of non-specific reaction.

b. Please confirm the optimal dilution ratio with the data sheet of the Neuropilin-1 (Mouse) ELISA kit.

4. Washing

Wash the wells 4 times with Wash solution following the Washing Method.

5. SA-HRP incubation

1) Add 100 µL of diluted SA-HRP solution to each well.

2) Incubate for 30 minutes at room temperature.

Note: Avoid touching the inner wall of the wells with the pipette tip. Otherwise, it could cause of non-specific reaction.

6. Washing

Wash the wells 4 times with Wash solution following the Washing Method.

7. Substrate incubation

Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature.

8. Stopping reaction

Add 100 µL of Stop Solution to each well.

9. Reading

Using microplate reader, read the absorbance of each well at 450 nm and the reference at 620 nm.

Note: a. Read absorbance within 30 minutes after reaction stop.

b. Ensure that the back of the plate is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before reading.

Calculation

Calculate the mean absorbance value of each standard and plot against log standard concentration and connect the points the best fitting straight line. The concentration of the samples then can be read from this standard curve. Alternatively a suitable computer and curve-fitting program can be used. The concentration read from the standard curve must be multiplied by the dilution factor.

Note: 1. If a sample's O.D. is out of range for the standard curve, the assay should be repeated with a higher sample dilution. ODs should always remain below 2.0 in order to remain in the dynamic range of the detection system.

2. When computer software is used, logistic, 3 (4) -para-logit-log, or Spline may be used as calculation.

Precautions

1. Do not use reagents beyond the stated expiration dates.
2. A standard curve must be run with each assay.
3. Operation for dispensing and diluting should be precisely done.
4. Avoid contact of Substrate Solution and Stop Solution with skin or eyes. If contacted, wash away with plenty of water.
5. Substrate Solution is easily oxidized with metal ions. Use disposable new instrument and disposable pipettes for all handling of the substrate solution NEVER return Substrate Solution to the bottle.
6. Serum samples may be infectious. Instruments used in this test should be disposed after use or treated as follows:

   Soak in 2% glutaraldehyde solution (final concentration) for more than one hour.

   Soak in 0.5% sodium hypochlorite solution (available chloric: approximately 5,000 ppm.) for more than one hour.

   Autoclave at 121°C for more than 20 minutes.
7. The incubation times indicated do not allow the incubation to complete. Frequent moving of the plate during standing or vibration from instruments, may cause a shaking effect to the reaction solution, causing the reaction to progresses faster than usual and giving higher color development.