Version 27-04/24

User's Manual

LRRK2 ELISA Kit (Human)

REF **DEIA-FN840NS**

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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

For the quantitative measurement of LRRK2 in serum, plasma, tissue homogenates, cell culture supernatants, and other biological tissues.

General Description

Positively regulates autophagy through a calcium-dependent activation of the CaMKK/AMPK signaling pathway. The process involves activation of nicotinic acid adenine dinucleotide phosphate (NAADP) receptors, increase in lysosomal pH, and calcium release from lysosomes. Together with RAB29, plays a role in the retrograde trafficking pathway for recycling proteins, such as mannose 6 phosphate receptor (M6PR), between lysosomes and the Golgi apparatus in a retromer-dependent manner. Regulates neuronal process morphology in the intact central nervous system (CNS). Plays a role in synaptic vesicle trafficking. Phosphorylates PRDX3. Has GTPase activity. May play a role in the phosphorylation of proteins central to Parkinson disease. Plays an important role in recuiting SEC16A to endoplasmic reticulum exit sites (ERES) and in regulating ER to Golgi vesicle-mediated transport and ERES organization.

Principles of Testing

CD LRRK2 ELISA Kit (Human) is based on standard sandwich enzymelinked immuno-sorbent assay technology. An antibody specific for LRRK2 has been pre-coated onto a 96-well plate (12 × 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for LRRK2 is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP to generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample LRRK2 captured in the well.

Reagents And Materials Provided

1. Anti-LRRK2 Microplate, 96 Wells (12×8 Well strips), -20°C for 6 months
2. LRRK2 Lyophilized Standard, 2×20 ng, -20°C for 6 months
3. 100× Biotinylated LRRK2 Detector Antibody, 1×120 µL, -20°C for 6 months
4. 100× Avidin-HRP Conjugate, 1×120 µL, -20°C for 6 months
5. Sample Diluent, 1×20 mL, -20°C for 6 months
6. Detector Antibody Diluent, 1×12 mL, -20°C for 6 months
7. Conjugate Diluent, 1×12 mL, -20°C for 6 months
8. 25× Wash Buffer, 1×30 mL, Store at 4°C for 6 months
9. Stop Solution, 1×10 mL, Store at 4°C for 6 months
10. TMB Substrate, 1×10 mL, Store at 4°C for 6 months

Materials Required But Not Supplied

1. Microplate reader capable of reading absorbance at 450 nm.
2. Automated plate washer (optional).
3. Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
4. Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
5. New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
6. Absorbent paper or paper toweling.
7. Distilled or deionized ultrapure water.
8. 37°C Incubator (optional)

Storage

Open kit immediately upon receipt. Store components at -20°C (**NOTE: exceptions below**) for 6 months or until expiration date. Avoid any freeze/thaw cycles.

Specimen Collection And Preparation

Sample Preparation and Storage

Store samples to be assayed at 4°C for 24 hours prior being assayed.

For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Samples not indicated in the manual must be tested to determine if the kit is valid.

Prepare samples as follows:

1. Serum: Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

2. Plasma: Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

3. Tissue Homogenates: Rinse 100 mg tissue with 1× PBS then homogenize in 1 mL of 1× PBS and store overnight at -20°C. Perform two freeze-thaw cycles to lyse the cell membranes, then centrifuge the homogenates for 5 minutes at 5,000 x g, 4°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

4. Cell culture supernatants 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.

Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting

saturation should be further diluted.

1. Dilute samples using Sample Diluent.
2. Mix diluted samples gently and thoroughly.
3. Pipetting less than 2 µL is not recommended for optimal assay accuracy.

Plate Preparation

1. Micro-plates are provided ready to use and do not require rinsing or blocking.
2. Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
3. Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

Reagent Preparation

Equilibrate all materials to room temperature prior to use and use immediately.

1. LRRK2 Assay Standards

a. Prepare the LRRK2 standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

b. Reconstitute one vial of the provided 20 ng Lyophilized LRRK2 Standard for each experiment. Prepare the stock 10 ng/mL Standard by reconstituting one tube of Lyophilized LRRK2 Standard as follows:

Gently spin or tap the vial at 6,000-10,000 rpm for 30 seconds to collect all material at the bottom.

Add 2 mL of Sample Diluent to the vial.

Seal the vial then mix gently and thoroughly.

Leave the vial at ambient temperature for 15 minutes.

c. Prepare a set of seven serially diluted standards as follows:

Label tubes with numbers 2-8.

Use the reconstituted 10 ng/mL LRRK2 Standard as the high standard point (Tube #1).

Add 300 µL of Sample Diluent to Tube #'s 2-8.

Prepare Standard #2 by adding 300 µL of 10 ng/mL LRRK2 (Tube #1) to Tube #2. Mix gently and thoroughly.

Prepare Standard #3 by adding 300 µL of Standard #2 from Tube #2 to Tube #3. Mix gently and thoroughly.

Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

Tube #8 is a blank standard (only Sample Diluent), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Sample Diluent (µL)	Total Volume (µL)	Final Concentration
1	20 ng/mL Reconstituted LRRK2 Standard	NA	2,000	2,000	10 ng/mL
2	10 ng/mL	300	300	600	5 ng/mL
3	5 ng/mL	300	300	600	2.5 ng/mL
4	2.5 ng/mL	300	300	600	1.25 ng/mL
5	1.25 ng/mL	300	300	600	0.625 ng/mL
6	0.625 ng/mL	300	300	600	0.312 ng/mL
7	0.312 ng/mL	300	300	600	0.156 ng/mL
8	NA	0	300	300	0.0 (Blank)

2. 1× Biotinylated LRRK2 Detector Antibody

a. Prepare the 1× Biotinylated LRRK2 Detector Antibody immediately prior to use by diluting the 100× Biotinylated LRRK2 Detector Antibody 1:100 with Detector Antibody Diluent.

b. For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of 100× Biotinylated LRRK2 Detector Antibody to 990 µL Detector Antibody Diluent.

c. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1× concentration for future use.

3. 1× HRP-Avidin Conjugate

a. Prepare the 1× Avidin-HRP Conjugate immediately prior to use by diluting the 100X Avidin-HRP Conjugate 1:100 with Conjugate Diluent.

b. For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of 100× Avidin-HRP Conjugate to 990 µL Conjugate Diluent.

c. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1× concentration for future use.

4. 1× Wash Buffer

a. If crystals have formed in the 25× Wash Buffer concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

b. Add the entire 30 mL contents of the 25× Wash Buffer bottle to 720 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

c. Seal and mix gently by inversion. Avoid foaming or bubbles.

d. Store the 1× Wash Buffer at room temperature until ready to use in the procedure. Store the prepared 1× Wash Buffer at 4°C for no longer than 1 week. Do not freeze.

Assay Procedure

Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

Optionally, to control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

1. Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.
2. Add 100 µL of serially titrated standards, diluted samples or blank into wells of the Anti-LRRK2 Microplate. At least two replicates of each standard, sample or blank is recommended.
3. Cover the plate with the plate sealer and incubate for 2 hours.
4. Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
5. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
6. Add 100 µL of prepared 1× Biotinylated LRRK2 Detector Antibody to each well.
7. Cover with the plate sealer and Incubate for 60 minutes.
8. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
9. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
10. Wash plate 3 times with 1× Wash Buffer as follows:

    a. Add 300 µL of 1X Wash Buffer to each assay well.

    b. Incubate for 1 minute.

    c. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

    d. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

    Repeat steps **a.** through **b.** two more times.
11. Add 100 µL of prepared 1× Avidin-HRP Conjugate into each well, cover with plate sealer and incubate for 60 minutes.
12. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
13. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
14. Wash plate 5 times with 1× Wash Buffer as in Step 10.
15. Add 90 µL of TMB Substrate to each well, cover with plate sealer and incubate in the dark for 15-30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time. (**NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.**)
16. Add 50 µL of Stop Solution to each well. Well color should change to yellow immediately. Add the Stop

Solution in the same well order as done for the TMB Substrate.

17. Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 16. If wavelength correction is available, set to 540 nm or 570 nm.

Calculation

For analysis of the assay results, calculate the Relative OD450 for each test or standard well as follows:

(Relative OD450) = (Well OD450)–(Mean Blank Well OD450)

The standard curve is generated by plotting the mean replicate Relative OD450 of each standard serial dilution point vs. the respective standard concentration. The LRRK2 concentration contained in the samples can be interpolated by using linear regression of each mean sample Relative OD450 against the standard curve. This is best achieved using curve fitting software.

Note: If wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

Specificity

No detectable cross-reactivity with other relevant proteins