# ab313910 – Human Nogo-B/RTN 4B ELISA Kit

For the quantitative measurement of Nogo-B/RTN 4B in human serum, plasma and cell culture supernatants.

For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:**
**http://www.abcam.com/ab313910**

## Storage and Stability:

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| 150X HRP-Streptavidin | 200 µl | -20°C |
| 20X Wash Buffer | 25 mL | -20°C |
| 5X Assay Diluent (Item E2) | 15 mL | -20°C |
| Stop Solution; 0.2M Sulfuric Acid | 8 mL | -20°C |
| TMB One-Step Substrate Reagent | 12 mL | -20°C |
| Human Nogo-B/RTN 4B Antibody-coated ELISA Plate | 1 unit | -20°C |
| Biotinylated Human Nogo-B/RTN 4B Detection Antibody | 2 vials | -20°C |
| Lyophilized Human Nogo-B/RTN 4B Protein Standard | 2 vials | -20°C |

## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µl to 1 ml volumes.
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

## Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

**5X Assay Diluent:** Dilute 5X Assay Diluent (Item E2) 5-fold with deionized or distilled water before use. 1X Assay Diluent (E2) should be used to dilute serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-fold.
**Note**: Levels of Nogo-B/RTN 4B may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

**Biotinylated Human Nogo-B/RTN 4B Detection Antibody:** Briefly spin the vial before use. Add 100 µL of 1X Assay Diluent (Item E2) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent (Item E2).

**20X Wash Buffer:** If the 20X Wash Buffer contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

**HRP-Streptavidin Concentrate**: Briefly spin the vial of 400X HRP-Streptavidin concentrate before use. HRP-Streptavidin should be diluted 150-fold with 1X Assay Diluent (Item E2).

For example: Briefly spin the vial and pipette up and down to mix gently. Add 80 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent (Item E2) to prepare a 150-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

## Standard Preparation

Always prepare a fresh set of standards for every use.
Discard working standard dilutions after use as they do not store well.
The following section describes the preparation of a standard curve for duplicate measurements (recommended).

- Briefly spin the Lyophilized Human Nogo-B/RTN 4B Protein Standard vial.
- Add 400 µl 1X Assay Diluent (Item E2, Assay Diluent should be diluted 5-fold with deionized or distilled water before use) into the Lyophilized Human Nogo-B/RTN 4B Protein Standard vial to prepare a 37500 pg/ml standard solution. Gently mix the powder to allow it to dissolve thoroughly.
- Add 40 µl of the 37500 pg/ml standard solution from the Lyophilized Human Nogo-B/RTN 4B Protein Standard vial into a tube with 460 µl 1X Assay Diluent (Item E2) to prepare a 3000 pg/ml standard solution.
- Pipette 300 µl 1X Assay Diluent (Item E2) into each tube.
- Use the 3000 pg/ml Standard solution to produce a dilution series (shown below). Adding 200 µL from Tube #1 to #2, then from #2 to #3, etc
- Mix each tube thoroughly before the next transfer.
- Tube #8 contains no protein and is the Blank control

| Tube # | Volume to dilute | Volume of 1X Assay Diluent | Final Concentration pg/mL |
|---|---|---|---|
| 1 | 3000 pg/ml standard stock solution | --- | 3000 |
| 2 | 200 µL of tube #1 | 300 µL | 1200 |
| 3 | 200 µL of tube #2 | 300 µL | 480 |
| 4 | 200 µL of tube #3 | 300 µL | 192 |
| 5 | 200 µL of tube #4 | 300 µL | 76.8 |
| 6 | 200 µL of tube #5 | 300 µL | 30.72 |
| 7 | 200 µL of tube #6 | 300 µL | 12.29 |
| 8 | -- | 300 µL | 0 |

## Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use. We recommend that you assay all standards, controls and samples in duplicate. Prepare all reagents, working standards, and samples as directed in the previous sections. **Note:** 1X Assay Diluent should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-fold.

1. Label removable 8-well strips as appropriate for your experiment.

2. Add 100 µl of each standard and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.

3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of 1X prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 µl of prepared Streptavidin solution to each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 3.

8. Add 100 µL of TMB Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

## Calculation of results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

**Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:** www.abcam.com/protocols/the-complete-elisa-guide

**For technical support contact information, visit:** www.abcam.com/contactus

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