Instructions for Use

For the quantitative measurement of TrkB in mouse cell and tissue extracts.

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

1. BACKGROUND

Abcam’s TrkB in vitro SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of TrkB protein in mouse cell and tissue extracts.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

TrkB is a tyrosine kinase receptor involved in the development of the peripheral and central nervous system. TrkB plays a role in synapse plasticity and formation, and neuron survival, proliferation and differentiation. Ligand (including BDNF and NTF4) interactions induces homodimerization, autophosphorylation and activation of TrkB. Activity of TrkB is also affected by the formation of inactive heterodimers with noncatalytic isoforms.
2. **ASSAY SUMMARY**

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.

Add standard or sample to appropriate wells.

Add Antibody Cocktail to all wells. Incubate at room temperature.

Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.
PDF Text

3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent and Standard Preparation sections.

5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse TrkB Capture Antibody (Lyophilized)</td>
<td>1 Vial</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>10X Mouse TrkB Detector Antibody</td>
<td>600 µL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Mouse TrkB Lyophilized Recombinant Protein</td>
<td>2 Vials</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Antibody Diluent 5BI</td>
<td>6 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>10X Wash Buffer PT</td>
<td>20 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>5X Cell Extraction Buffer PTR</td>
<td>10 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>50X Cell Extraction Enhancer Solution</td>
<td>1 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>12 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Sample Diluent NS</td>
<td>12 mL</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Pre-Coated 96 Well Microplate (12 x 8 well strips)</td>
<td>96 Wells</td>
<td>+2-8°C</td>
</tr>
<tr>
<td>Plate Seal</td>
<td>1</td>
<td>+2-8°C</td>
</tr>
</tbody>
</table>
6. **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 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. **TECHNICAL HINTS**

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
• Ensure plates are properly sealed or covered during incubation steps.
• Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
• As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
• All samples should be mixed thoroughly and gently.
• Avoid multiple freeze/thaw of samples.
• Incubate ELISA plates on a plate shaker during all incubation steps.
• When generating positive control samples, it is advisable to change pipette tips after each step.
• The provided 5X Cell Extraction Buffer contains phosphatase inhibitors and protease inhibitor aprotinin. Additional protease inhibitors can be added if required.
• The provided Antibody Diluents and Sample Diluents contain protease inhibitor aprotinin. Additional protease inhibitors can be added if required.
• The provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4ºC. To dissolve, warm briefly at + 37ºC and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.
• To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
• This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
9. **REAGENT PREPARATION**

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 **1X Cell Extraction Buffer PTR**

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution. Mix thoroughly and gently. If required protease inhibitors can be added.

*Alternative* – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

9.2 **10X Capture Antibody**

Prepare the 10X Capture Antibody solution by adding 660 µL Sample Diluent NS to the lyophilized capture antibody. Rotate at room temperature for 10 minutes to mix thoroughly.

9.3 **1X Wash Buffer PT**

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.4 **Antibody Cocktail**
Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.
10. **STANDARD PREPARATION**

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following table describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the mouse TrkB lyophilized protein standard sample by adding 100 µL water by pipette. Mix thoroughly and gently. Hold at room temperature for 3 minutes and mix gently. This is the 50,000 pg/mL Stock Standard Solution.

10.2 Label eight tubes, Standards 1–8.

10.3 Add 360 µL 1X Cell Extraction Buffer PTR into tube number 1 and 1X Cell Extraction Buffer PTR µL of 150 into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:
11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse Brain Homogenate</td>
<td>12.5 - 50 µg/mL</td>
</tr>
</tbody>
</table>

11.1 Preparation of extracts from cell pellets

11.1.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.1.2 Rinse cells twice with PBS.

11.1.3 Solubilize pellet at 2x10⁷ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.1.4 Incubate on ice for 20 minutes.

11.1.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.1.6 Transfer the supernatants into clean tubes and discard the pellets.

11.1.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.1.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.2 Preparation of extracts from adherent cells by direct lysis (alternative protocol)

11.2.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.2.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).
11.2.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.2.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.2.5 Transfer the supernatants into clean tubes and discard the pellets.

11.2.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.2.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.3 Preparation of extracts from tissue homogenates

11.3.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.3.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.3.3 Incubate on ice for 20 minutes.

11.3.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.3.5 Transfer the supernatants into clean tubes and discard the pellets.

11.3.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.3.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.
12. **PLATE PREPARATION**

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.
13. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Substrate to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:
### ASSAY PROCEDURE

<table>
<thead>
<tr>
<th>Mode</th>
<th>Kinetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>600 nm</td>
</tr>
<tr>
<td>Time</td>
<td>up to 15 min</td>
</tr>
<tr>
<td>Interval</td>
<td>20 sec - 1 min</td>
</tr>
<tr>
<td>Shaking</td>
<td>Shake between readings</td>
</tr>
</tbody>
</table>

*Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.*

13.9 Analyze the data as described below.
14. **CALCULATIONS**

Subtract the average zero standard absorbance value from all measurements to determine the background-corrected absorbance value. Determine the average value of the duplicate background-subtracted measurements of the positive control dilutions and plot these values against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Software supplied with microplate readers have or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to determine the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). The concentrations of unknown samples can be interpolated from the curve using the background-corrected absorbance values and the dilution factor, if used. Absorbance values of samples which are greater than that of the highest standard should be diluted until the absorbance value falls within the linear part of the curve. The concentration of the sample can then be determined by applying the dilution factor.
15. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

<table>
<thead>
<tr>
<th>Conc. (pg/mL)</th>
<th>O.D. 450 nm</th>
<th>Mean O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>0.047</td>
<td>0.046</td>
</tr>
<tr>
<td>78</td>
<td>0.069</td>
<td>0.064</td>
</tr>
<tr>
<td>156</td>
<td>0.084</td>
<td>0.083</td>
</tr>
<tr>
<td>313</td>
<td>0.122</td>
<td>0.116</td>
</tr>
<tr>
<td>625</td>
<td>0.237</td>
<td>0.242</td>
</tr>
<tr>
<td>1,250</td>
<td>0.548</td>
<td>0.544</td>
</tr>
<tr>
<td>2,500</td>
<td>1.392</td>
<td>1.419</td>
</tr>
<tr>
<td>5,000</td>
<td>3.368</td>
<td>3.430</td>
</tr>
</tbody>
</table>

**Figure 1.** Example of mouse TrkB standard curve. The TrkB standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.
16. **TYPICAL SAMPLE VALUES**

**SENSITIVITY –**

The calculated minimal detectable dose (MDD) is 33.3 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=25) and adding 2 standard deviations then extrapolating the corresponding concentrations.

**RECOVERY –**

Three concentrations of TrkB were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5 µg/mL Mouse Brain Extract</td>
<td>106</td>
<td>103 - 110</td>
</tr>
</tbody>
</table>
LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native TrkB was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

<table>
<thead>
<tr>
<th>Dilution Factor</th>
<th>Interpolated value</th>
<th>50 µg/mL Mouse Brain Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>pg/mL</td>
<td>1477</td>
</tr>
<tr>
<td></td>
<td>% Expected value</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>pg/mL</td>
<td>755</td>
</tr>
<tr>
<td></td>
<td>% Expected value</td>
<td>102</td>
</tr>
<tr>
<td>4</td>
<td>pg/mL</td>
<td>369</td>
</tr>
<tr>
<td></td>
<td>% Expected value</td>
<td>100</td>
</tr>
</tbody>
</table>

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of TrkB within the working range of the assay.

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.4</td>
<td>3.2</td>
</tr>
</tbody>
</table>
**Figure 2.** Specificity of mouse tissue samples within the working range of the assay. Interpolated values of TrkB are plotted based on an extract load of 50 µg/mL. Background-subtracted data values (mean +/- SD, n = 2) are graphed.
17. **ASSAY SPECIFICITY**

This kit recognizes both native and recombinant mouse TrkB protein in cell and tissue extract samples only.

No detectable levels were observed in serum, plasma and cell culture supernatant samples.

18. **SPECIES REACTIVITY**

This kit recognizes mouse and rat TrkB protein.

Rat brain extract was tested with this kit and was found to be cross reactive.

![Graph showing OD (450 nm) vs Extract (µg/mL)](image)

**Figure 3.** Rat and mouse brain extract were prepared as described in Section 11 and diluted 2-fold in 1X Cell Extraction Buffer PTR and assayed for cross reactivity. Interpolated values from the mouse protein standard are graphed.

Please contact our Scientific Support team for more information
## 19. **TROUBLESHOOTING**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficulty pipetting lysate; viscous lysate.</td>
<td>Genomic DNA solubilized</td>
<td>Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.</td>
</tr>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate Pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standard dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td></td>
<td>Incubation times with TMB too brief</td>
<td>Ensure sufficient incubation time until blue color develops prior addition of Stop solution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the ELISA kit</td>
<td>Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.</td>
</tr>
<tr>
<td>Precipitate in Diluent</td>
<td>Precipitation and/or coagulation of components within the Diluent.</td>
<td>Precipitate can be removed by gently warming the Diluent to 37°C.</td>
</tr>
</tbody>
</table>
20. **NOTES**
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