Mouse BDNF/TNF/NGF/IL1b/IL18/ACE2 Multiplex ELISA Kit

Catalog No: abx481100

Size: 96T

Analyte	Range	Sensitivity
BDNF	0.483 pg/ml – 500 pg/ml	< 0.16 pg/ml
TNF	0.977 pg/ml – 1000 pg/ml	< 0.33 pg/ml
NGF	0.977 pg/ml – 1000 pg/ml	< 0.33 pg/ml
IL1b	0.977 pg/ml – 1000 pg/ml	< 0.33 pg/ml
IL18	0.977 pg/ml – 1000 pg/ml	< 0.33 pg/ml
ACE2	0.781 pg/ml – 800 pg/ml	< 0.26 pg/ml

Storage: Store the 96-well plate, Standards, Standard Diluent, Detection Reagent A, and Diluent A at -20°C, and the rest of the kit components at 4°C. Magnetic Beads and Detection Reagent B should be stored in the dark.

Application: The quantitative detection of BDNF, TNF, NGF, IL1b, IL18, ACE2 in Mouse serum, plasma, tissue homogenates, cell lysates, cell culture supernatant, and other biological fluids.

Principle of the Assay: This assay is based on Flow Luminescence Immunoassay (FLIA) technology using bead-based multiplex detection. Antibodies specific to the desired analyte(s) are pre-coated onto magnetic microsphere beads, which are internally dyed and correspond to a unique fluorescent bead region. Standards and test samples are added to the bead mixture, where target analytes bind to their corresponding capture antibodies, followed by incubation. Biotinylated detection antibodies are added to form an antibody-antigen-antibody sandwich. After incubation, phycoerythrin (PE)-labeled avidin is added, which binds to the biotinylated detection antibodies. Unbound conjugated are removed using wash buffer following each incubation.

Beads are analyzed using a flow cytometer equipped with dual lasers. One laser identifies the bead region and its corresponding analyte. The second laser quantifies the PE signal, which is directly proportional to the amount of analyte bound. The concentration of each analyte is determined by comparing the Median Fluorescence Intensity (MFI) to the standard curve.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Standard: 2 tubes
- Magnetic Beads: 1 ml
- Wash Buffer: (30X) 20 ml
- Standard Diluent Buffer: 20 ml
- Analysis Buffer: 20 ml
- Detection Reagent A: (100X) 120 µl
- Detection Reagent B: (100X) 120 µl
- Diluent A: 12 ml
- Diluent B: 12 ml
- Sheath Fluid: 10 ml
- Plate Sealer: 4

Materials Required But Not Provided

- 37°C incubator with microplate shaker
- Multi and single channel pipettes and sterile pipette tips
- Microcentrifuge tubes
- Distilled water
- Vortex mixer
- 0.01 mol/L PBS (pH 7.0 - 7.2)
- Multiplex microplate reader
- Squirt bottle and magnetic separator for 96-well microplate (or an automated magnetic microplate washer)

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Protocol

A. Sample Preparation

Analyze immediately or store samples at 2 - 8°C for up to 1 week. For long term storage, aliquot and store at -20°C for up to 1 month or -80°C for up to 2 month. Avoid multiple freeze-thaw cycles. Keep samples on ice during preparation. Equilibrate samples to room temperature prior to analysis.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. Collect the supernatant and centrifuge at 10,000 × g for 20 - 30 mins at 2 - 8°C. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge at 1000 × g for 15 mins at 2 - 8°C, within 30 mins of collection. Collect the supernatant and centrifuge at 10,000 × g for 20 - 30 mins at 2 - 8°C. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C. Avoid hemolytic samples.
- **Tissue Homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 10,000 × g for 20 - 30 mins at and collect the supernatant. Assay immediately or aliquot and store at -20°C or -80°C.
- **Cell Lysates:** Detach adherent cells with trypsin and collect by centrifugation and remove the supernatant. Wash the cells three times in ice-cold PBS and re-suspend cells in PBS at a concentration of 107 cells/ml. Lyse the cells by ultra-sonification until the solution is clear. Centrifuge at 10,000 × g for 10 mins at 2 - 8°C to remove cellular debris. Collect the supernatant. Assay immediately or aliquot and store at -20°C or -80°C.
- **Cell Culture Supernatant:** Centrifuge at approximately 10,000 × g for 20 - 30 mins at 2 - 8°C to remove precipitate. Collect the supernatant and assay immediately or store at -20°C or -80°C.
- **Other Biological Fluids:** Centrifuge at approximately 10,000 × g for 20 - 30 mins at 2 - 8°C to remove precipitate. Collect the supernatant and assay immediately or store at -20°C or -80°C.

Notes:

- **Frozen samples should be centrifuged at 10,000 × g for 20 - 30 mins at 2 - 8°C after thawing. Collect the supernatant and assay immediately.**
- Samples must be diluted so that the expected concentration falls within the kit's range. The sample should be diluted in 0.01 mol/L PBS (PH=7.0-7.2).
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

B. Reagent Preparation

Standard Dilutions: Reconstitute the Standard with 0.5 ml of Standard Diluent Buffer to make the highest standard solution (Standard 1). Allow the reconstituted standard to sit for 10 mins, with gentle agitation prior to carrying out the serial dilutions. Avoid foaming or bubbles. Label 6 tubes from Standard 2 – Standard 6 in preparation for the serial dilutions - *see the diagram and table below for reference*. Aliquot 0.3 ml of the Standard Diluent Buffer into each tube (apart from the highest standard tube). Add 0.1 ml of the highest standard solution into the Standard 2 tube and mix thoroughly. Transfer 0.1 ml from the Standard 2 tube to the Standard 3 tube, mix thoroughly, and so on.

Note: Do not vortex the standard during reconstitution, as this will destabilize the protein. Once the standard has been reconstituted, it should be used within 15 mins. It is not recommended to reuse the reconstituted standard.

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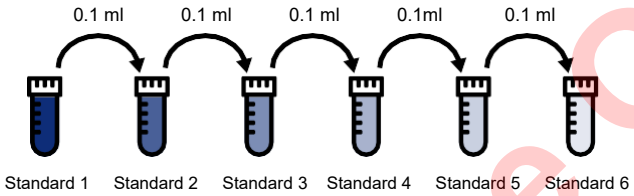
Analyte	Standard Concentration (pg/ml)					
	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
BDNF	500	125	31.3	7.81	1.95	0.49
TNFa	1000	250	62.5	15.6	3.91	0.98
NGF	1000	250	62.5	15.6	3.91	0.98
IL1b	1000	250	62.5	15.6	3.91	0.98
IL18	1000	250	62.5	15.6	3.91	0.98
ACE2	800	200	50	12.5	3.13	0.78



Wash Buffer: Dilute the concentrated Wash Buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Detection Reagent A Working Solution Preparation: Prepare no more than 15 mins before the experiment.

1. Calculate the total volume of working solution required.
2. Dilute Detection Reagent A 100-fold with Diluent A, and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

Detection Reagent B Working Solution Preparation: Prepare no more than 15 mins before the experiment.

1. Calculate the total volume of working solution required.
2. Dilute Detection Reagent B 100-fold with Diluent B, and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

C. Assay Protocol

Prepare all standards, samples and reagents as directed above. Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate, and to plot a standard curve for each test.

1. Assign and record microplate wells for each standard, sample and control (zero).
2. Add 200 µl of Analysis Buffer to each well. *Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
3. Put the plate on a plate shaker at 800 rpm with an amplitude of 2 - 4 mm for 10 mins, then discard the Buffer Solution from each well.
4. Add 100 µl of Standard Dilutions into the standard wells.
5. Add 100 µl of Standard Diluent Buffer into the control (zero) wells.
6. Add 100 µl of appropriately diluted sample into the sample wells.
7. Briefly vortex the Magnetic Beads, then immediately add 10 µl of Magnetic Beads to each well. Cover the plate with a plate sealer.
8. **For tissue homogenate samples:** incubate for 2 hrs at 18 - 25°C in the dark, on a plate shaker at 800 rpm with an amplitude of 2 - 4 mm. **For all other sample types:** incubate for 90 mins at 37°C in the dark, on a plate shaker at 800 rpm with an amplitude of 2 - 4 mm.
9. Put the plate on the magnetic separator for 2 mins, then discard the liquid from each well. *Do not wash.*
10. Add 100 µl of Detection Reagent A Working Solution to each well. Cover the plate with a plate sealer and incubate for 1 hr at 37°C in the dark, on a plate shaker at 800 rpm with an amplitude of 2 - 4 mm.

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11. Put the plate on the magnetic separator for 2 mins, then discard the solution from each well.
12. Keeping the plate on the magnetic separator, wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (200 µl) using a multi-channel Pipette or automated microplate washer (1 - 2 mins soaking period is recommended). *Complete removal of liquid at each step is essential for good performance. Do not blot the plate on absorbent paper.*
13. Remove the plate from the magnetic separator and add 100 µl of Detection Reagent B Working Solution to each well. Seal the plate with a plate sealer and incubate for 30 mins at 37°C in the dark, on a plate shaker at 800 rpm with an amplitude of 2 - 4 mm.
14. Put the plate on the magnetic separator for 2 mins, discard the solution from each well and repeat the wash procedure as described in step 12.
15. Remove the plate from the magnetic separator and add 100 µl of Sheath Fluid to each well.
16. Cover the plate with a plate sealer and incubate for 10 mins at 37°C in the dark, on a plate shaker at 800 rpm with an amplitude of 2 - 4 mm.
17. Read the plate with a flow cytometer with the settings as described below.

Flow Cytometer	
Analyte Volume	50 µl
Fluorochromes	APC, APC-Cy7, PE
Total Events	100 × number of targets
Median Fluorescence Intensity (MFI)	Median

Calculate results automatically using the flow cytometer, or calculate manually by averaging the MFI readings for each reference standard and sample, then subtracting the average control (zero) MFI reading.

(Relative MFI) = (MFI of Each Well) – (MFI of Zero Well)

The standard curve can be plotted as the relative MFI of each reference standard solution (X), against the respective concentration of each standard solution (Y). The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the concentrations from interpolation by the dilution factor, to obtain the concentration before dilution.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, protected from light, and that the time and temperature are controlled.
- Do not allow the microplate wells to dry out at any point during the assay.
- Use of a magnetic separator during wash steps and when discarding solution is essential to prevent the loss of magnetic beads. Do not blot the plate on absorbent paper.
- Ensure the needle height of the instrument is correct before reading the plate. The needle height should be 2 magnetic gaskets from the bottom of the plate wells.
- If the field strength of the magnetic separator is less than 5000 Gauss, the magnetic attraction time should be extended to 4 mins.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- Please note that this kit is optimized for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

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Technical Support

For troubleshooting, frequently asked questions, and assistance, please visit:
https://www.abbexa.com/scientific-support/troubleshooting-and-faqs/elisa-kit-scientific-support or email us at support@abbexa.com.

Precision:

- Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of BDNF, TNF, NGF, IL1b, IL18 and ACE2 were tested 20 times on one plate, respectively.
- Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of BDNF, TNF, NGF, IL1b, IL18 and ACE2 were tested on 3 different plates, 8 replicates in each plate.
- CV (%) = (Standard Deviation / Mean) × 100
  Intra-Assay: CV < 10%
  Inter-Assay: CV < 12%

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