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Antibodies | ELISA kits | Proteins

# Human/Rat BDNF sandwich ELISA kit datasheet

For the quantitative detection of human/rat BDNF in serum, plasma and cell culture supernatants.

## general information

| | |
|---|---|
| **Catalogue Number** | KE00096 |
| **Product Name** | Human/Rat BDNF ELISA Kit |
| **Species cross-reactivity** | Human/Rat BDNF |
| **Range (calibration Range)** | 12.5 - 800 pg/mL |
| **Tested applications** | Quantification ELISA |

## database links

| | |
|---|---|
| **Entrez Gene** | **627** (Human) / **24225** (Rat) |
| **SwissProt** | **P23560** (Human) / **P23363** (Rat) |

## kit components & storage

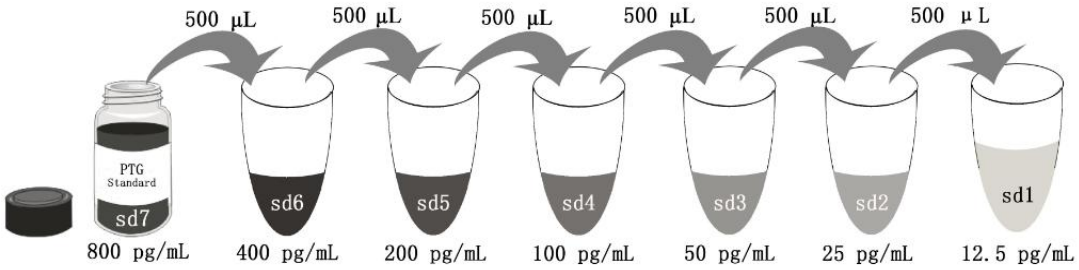
| | | |
|---|---|---|
| **Microplate** - antibody coated 96-well Microplate (8 well × 12 strips) | 1 plate | Store at 2-8°C for six months |
| **Standard** - 1600 pg/bottle; lyophilized* | 2 bottles | Store at 2-8°C for six months |
| **Detection Antibody (100X), biotinylated** - 120 µL/vial | 1 vial | Store at 2-8°C for six months |
| **Streptavidin-HRP antibody (100X)** - 120 µL/vial | 1 vial | Store at 2-8°C for six months |
| **Sample Diluent PT 1-ec** - 30 mL/bottle | 1 bottle | Store at 2-8°C for six months |
| **Detection Diluent** - 30 mL/bottle | 1 bottle | Store at 2-8°C for six months |
| **Wash Buffer Concentrate (20X)** - 30 mL/bottle | 1 bottle | Store at 2-8°C for six months |
| **Tetramethylbenzidine Substrate (TMB)** - 12 mL/bottle | 1 bottle | Store at 2-8°C for six months |
| **Stop Solution** - 12 mL/bottle | 1 bottle | Store at 2-8°C for six months |
| **Plate Cover Seals** | 3 pieces | |

**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 1-ec is for standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP antibody.

*Add **2 mL** Sample Diluent PT 1-ec in standard. This reconstitution gives a stock solution of 800 pg/mL.




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| # µL of Sample Diluent PT 1-ec | **2000 µL** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |

## product description

KE00096 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The BDNF ELISA kit is to be used to detect and quantify protein levels of endogenous BDNF. The assay recognizes human BDNF. An antibody specific for BDNF has been pre-coated onto the microwells. The BDNF protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human BDNF is added to detect the captured human BDNF protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm with the correction wavelength set at 630 nm.

## background

The brain-derived neurotrophic factor (BDNF), as a member of the neurotrophin family of growth factors, is broadly expressed in the central nervous system. BDNF is involved in cerebral morphogenesis, synaptic plasticity and learning, mental health, degenerative central nervous system (CNS) diseases, and energy metabolism. BDNF has recently been identified as one of the four major pharmaceutical targets in neuropsychiatric diseases, including depression and bipolar disorder. In addition, BDNF is increasingly considered as a potential therapeutic agent for neurodevelopmental disorders such as Rett syndrome.

## sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:50 is suggested according to the individual samples.

## safety notes

This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.



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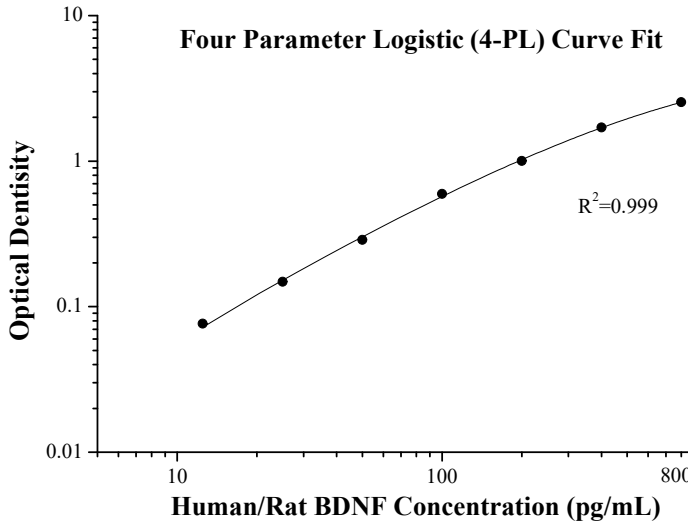
## assay procedure summary

| Step | Reagent | Volume | Incubation | Wash | Notes |
|---|---|---|---|---|---|
| 1 | Standard and Samples | 100 µL | **120 min** | 4 times | Cover Wells incubate at 37°C |
| 2 | Diluent Antibody Solution | 100 µL | 60 min | 4 times | Cover Wells incubate at 37°C |
| 3 | Diluent HRP Solution | 100 µL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 4 | TMB Substrate | 100 µL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 5 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 6 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |

## typical data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.038 | 0.035 | — |
| | 0.031 | | |
| 12.5 | 0.116 | 0.111 | 0.077 |
| | 0.106 | | |
| 25 | 0.164 | 0.183 | 0.149 |
| | 0.202 | | |
| 50 | 0.306 | 0.323 | 0.288 |
| | 0.339 | | |
| 100 | 0.622 | 0.631 | 0.596 |
| | 0.639 | | |
| 200 | 1.067 | 1.038 | 1.004 |
| | 1.009 | | |
| 400 | 1.615 | 1.737 | 1.703 |
| | 1.859 | | |
| 800 | 2.471 | 2.570 | 2.536 |
| | 2.669 | | |

## precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

| | Intra-assay Precision | | | Inter-assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 20 | 20 | 20 | 24 | 24 | 24 |
| Mean (pg/mL) | 35.8 | 116.5 | 513.9 | 32.2 | 108.6 | 517.3 |
| SD | 2.3 | 9.2 | 36.9 | 2.6 | 8.2 | 40.8 |
| CV% | 6.3 | 7.9 | 7.2 | 8.1 | 7.6 | 7.9 |



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## recovery

The recovery of BDNF spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

| Sample Type | | Average% of Expected | Range (%) |
|---|---|---|---|
| Human plasma | 1:50 | 91 | 74-110 |
| | 1:100 | 93 | 78-117 |
| Cell culture supernatants | 1:4 | 86 | 73-100 |
| | 1:8 | 94 | 75-113 |

## sample value

Serum samples from healthy volunteers (human) and normal rat were evaluated for BDNF in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean of Detectable (pg/mL) | Range (pg/mL) |
|---|---|---|
| Human serum (n=32) | 11,694 | 3,146-20,916 |
| Rat serum (n=8) | 117 | 89-177 |

## sensitivity

The minimum detectable dose of human BDNF is 0.1 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of BDNF in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:50)

| | | Human plasma | Cell culture supernatants |
|---|---|---|---|
| 1:2 | Average% of Expected | 82 | 78 |
| | Range (%) | 74-96 | 73-86 |
| 1:4 | Average% of Expected | 88 | 88 |
| | Range (%) | 75-109 | 79-94 |
| 1:8 | Average% of Expected | 97 | 91 |
| | Range (%) | 93-100 | 82-100 |
| 1:16 | Average% of Expected | 99 | 79 |
| | Range (%) | 76-111 | 70-87 |



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## references


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2. Autry A. et al. (2012) J. Neurosci. 32:14074-14079.
3. Yamada K. et al. (2003) J Pharmacol Sci. 91:267-70.
4. Vaghi V. et al. (2014) J Biol Chem. 289:27702-13.




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