

Pig BDNF ELISA Kit

Catalog Number: EA102785

Assay Principle

The OriGene Pig BDNF Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Pig BDNF with a 96-well strip plate that is pre-coated with antibody specific for BDNF. The detection antibody is a biotinylated antibody specific for BDNF. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Pig BDNF with immunogen: Expression system for standard: NSO; Immunogen sequence: H129-R247. The kit is analytically validated with ready to use reagents.

To measure Pig BDNF, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Pig BDNF in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Pig BDNF in the sample.

Overview

| | |
|-----------------------------|---|
| Product Name | Pig porcine BDNF ELISA Kit |
| Reactive Species | Pig |
| Size | 96wells/kit, with removable strips. |
| Description | Sandwich High Sensitivity ELISA kit for Quantitative Detection of Pig porcine BDNF. 96wells/kit, with removable strips. |
| Sensitivity | <15pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration. |
| Detection Range | 31.2pg/ml-2000pg/ml |
| Storage Instructions | Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.) |
| Uniprot ID | P23560 |

Technical Details

| | |
|-------------------------------------|---|
| Capture/Detection Antibodies | <i>The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.</i> |
| Specificity | <i>Natural and recombinant Pig BDNF</i> |
| Immunogen | <i>Expression system for standard: NSO; Immunogen sequence: H129-R247</i> |
| Cross Reactivity | <i>This kit is for the detection of pig BDNF. No significant cross-reactivity or interference between BDNF and its analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.</i> |

Notice Before Application

Please read the following instructions before starting the experiment.

1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see sections Sample Preparation) and that you have sufficient sample volume for the assay.
5. When first using the kit, appropriate validation steps should be taken before using valuable samples. Confirm kit adequately detects the antigen in your intended sample types by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see section Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
9. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
10. Don't reuse tips and tubes to avoid cross contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature and kit age can cause variation in binding. Variations in sample collection, processing and storage may cause sample value differences.

Kit Components/Materials Provided

| Description | Quantity | Volume |
|--|-----------------|-----------------------------|
| Anti-Pig BDNF Pre-coated 96-well strip microplate | 1 | 12 strips of 8 wells |
| Pig BDNF Standard | 2 | 10 ng/tube |
| Pig BDNF Biotinylated antibody (100x) | 1 | 100 µl |
| Avidin-Biotin-Peroxidase Complex (100x) | 1 | 100 µl |
| Sample Diluent | 1 | 30ml |
| Antibody Diluent | 1 | 12ml |
| Avidin-Biotin-Peroxidase Diluent | 1 | 12ml |
| Color Developing Reagent (TMB) | 1 | 10ml |
| Stop Solution | 1 | 10ml |
| Plate Sealers | 4 | Piece |
| Wash Buffer (25 x) | 1 | 20 ml |

Required Materials That Are Not Supplied

Microplate Reader capable of reading absorbance at 450nm.

Automated plate washer (optional)

Pipettes and pipette tips capable of precisely dispensing 0.5 μ l through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.

Deionized or distilled water.

500ml graduated cylinders.

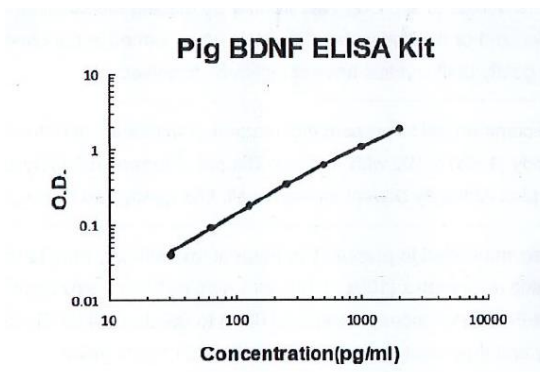
Test tubes for dilution.

Pig porcine BDNF ELISA Kit (EA102785) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

| Concentration (pg/ml) | 0 | 31.2 | 62.5 | 125 | 250 | 500 | 1000 | 2000 |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| O.D. | 0.033 | 0.073 | 0.125 | 0.213 | 0.376 | 0.658 | 1.120 | 1.905 |

Pig BDNF ELISA Kit standard curve



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

Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

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

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

| Sample | Intra-Assay Precision | | | Inter-Assay Precision | | |
|--------------------|-----------------------|---------|-------|-----------------------|-------|--------|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 16 | 16 | 16 | 24 | 24 | 24 |
| Mean(pg/ml) | 113 | 425 | 1278 | 197 | 594 | 1306 |
| Standard deviation | 3.96 | 114.185 | 62.62 | 14.18 | 44.55 | 103.17 |
| CV(%) | 3.5 | 4.2 | 4.9 | 7.2 | 7.5 | 7.9 |

Preparation Before The Experiment

| Item | Preparation |
|-------------------------------------|---|
| All reagents | Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C. |
| Wash buffer | Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved. |
| Biotinylated Anti-Pig BDNF antibody | It is recommended to prepare this reagent immediately prior to use by diluting the Pig BDNF Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation. |
| Avidin-Biotin-Peroxidase Complex | It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation. |
| Pig BDNF Standard | It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Pig BDNF standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions. |
| Microplate | The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging. |

Dilution of Pig BDNF Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 – 2000pg/ml, #2 – 1000pg/ml, #3 – 500pg/ml, #4 – 250pg/ml, #5 – 125pg/ml, #6 – 62.5pg/ml, #7 – 31.25pg/ml, #8 – 0.0 pg/ml (Blank – Sample Diluent serves as the blank).
2. To generate standard #1, add 200µl of the reconstituted standard stock solution of 10ng/ml and 800µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank standard to be used with every experiment.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

| Sample Type | Procedure |
|---------------------------|---|
| Cell culture supernatants | Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C. |
| Serum | Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C. |
| Plasma | Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site. |

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.

4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 μ l of the prepared 1x Biotinylated Anti-Pig BDNF antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
 - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 - b. Add 300 μ l of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
 - c. Repeat steps a-b 2 additional times.
9. Add 100 μ l of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
 - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 - b. Add 300 μ l of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
 - c. Repeat steps a-b 4 additional times.
11. Add 90 μ l of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 μ l of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on BDNF

Brain-derived neurotrophic factor (BDNF) is a prosurvival factor induced by cortical neurons that is necessary for survival of striatal neurons in the brain. It is a secreted protein with the molecular weight of 27.8kDa, consisting of 247 amino acids. It is known to promote neuronal survival and differentiation. BDNF shares substantial amino acid sequence identity with nerve growth factor (NGF). BDNF and neurotrophin-3 (NT-3) are two recently cloned neurotrophic factors that are homologous to NGF. mRNA products of the BDNF and NT-3 genes are detected in the adult Pig



OriGene Technologies Inc.

9620 Medical Center Drive, Suite 200, Rockville, MD 20850

Phone: 1.888.267.4436

Fax: 301-340-9254

Email: techsupport@origene.com

Web: www.origene.com

porcine brain, suggesting that these proteins are involved in the maintenance of the adult nervous system. BDNF and other neurotrophins are critically involved in long-term potentiation(LTP). BDNF-mediated LTP is induced postsynaptically. BDNF has trophic effects on serotonergic(5-HT) neurons in the central nervous system. BDNF has an essential maintenance function in the regulation of anxiety-related behavior and in food intake through central mediators in both the basal and fasted state. It plays a role in treating breathing disorders such as respiratory insufficiency after spinal injury. The mature form of BDNF is identical in all mammals examined, and the gene encoding Pig porcine BDNF to chromosome 11, band p13.