Mouse Brain Derived Neurotrophic Factor, BDNF GENLISA™ ELISA

REF : KLM0013

Ver 2.2

RUO

Enzyme Immunoassay for the Quantitative determination of BDNF in mouse serum and cell culture supernatant.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF **KLM0013** **Σ 96 tests**

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Introduction:

The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use:

The Mouse BDNF GENLISA™ ELISA kit is used as an analytical tool for quantitative determination of Mouse BDNF in mouse serum and other biological samples.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Mouse BDNF present in the sample are bound by the antibodies. Biotin labeled antibody is added and followed by Streptavidin-HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Mouse BDNF in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti-Mouse BDNF Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Mouse BDNF Standard (lyophilized, 140 ng/ml) – 2 vials
3. Biotinylated Anti Mouse BDNF Detection Antibody (lyophilized, 4.5 ug/ml) - 1 vial
4. Streptavidin:HRP - 1 vial
5. (1X) Assay Diluent – 50 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Store Standard and detection at 2-8°C. Upon reconstitution, aliquot standard and detection into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
4. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
5. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation:

1. **Please refer to lot specific instructions for preparation of the reagents.**

2. Standards Preparation: Reconstitute the lyophilized vial in 25ul of Assay Diluent to get 140 ng/ml standard concentrations. Add 10.72 ul of reconstituted standard to 989.28 ul of Assay Diluent (1X) to get a concentration of 1500 pg/ml. Perform serial dilutions as per table below. The Mouse BDNF standards are 1500 pg/ml, 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.75 pg/ml, 46.88 pg/ml and 23.44 pg/ml. Assay Diluent (1X) serves as Zero Standard (0 pg/ml).

Standard Concentration	Standard Vial	Dilution Particulars
140 ng/ml	Lyophilized Standard	Original Standard provided in the Kit + 25ul of Assay Diluent
1500 pg/ml	Standard No.7	10.72 ul Reconstituted Standard + 989.28 ul Assay Diluent (1X)
750 pg/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay Diluent (1X)
375 pg/ml	Standard No.5	500 ul Standard No.6 + 500 ul Assay Diluent (1X)
187.5 pg/ml	Standard No.4	500 ul Standard No.5 + 500 ul Assay Diluent (1X)
93.75 pg/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay Diluent (1X)
46.88 pg/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay Diluent (1X)
23.44 pg/ml	Standard No.1	500 ul Standard No.2 + 500 ul Assay Diluent (1X)
0 pg/ml	Standard No.0	Only Assay Diluent (1X)

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Mouse BDNF. High Dose Hook Effect is due to excess of antibody for very high concentrations of Mouse BDNF present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent.
3. Mouse BDNF concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.

4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Mouse BDNF.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. Bring all reagents to Room temperature before use. It is strongly recommended that all standards and Samples to be run in duplicates. A standard curve is required for each assay.

2. Add 100ul/well of **Standards and Samples** to the plate. Seal the plate and incubate for 2 hours at Room Temperature (18-25°C).

3. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

4. Add 100ul/well of **Detection Antibody** solution to each well. Seal the plate and incubate for 2 hours at Room Temperature (18-25°C).

5. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

6. Add 100µl of diluted **Streptavidin-HRP** solution to each well, seal plate and incubate for 30 minutes at Room Temperature (18-25°C).

7. Wash the plate 4 times with **Wash Buffer (1X)** as in Step 4**.**

8. Add 100µl of **TMB Substrate** solution and incubate in the dark for 30 minutes at RT. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.

9. Stop reaction by adding 100µl of **Stop Solution** to each well. Positive wells should turn from blue to yellow.

10. Read absorbance at 450 nm within 30 minutes of stopping reaction.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Mouse BDNF concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Mouse BDNF Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:
This kit has been validated. Please view the details herein below.

Standard Calibration Range:

23.44 pg/ml - 1500 pg/ml

Sensitivity:
Limit Of Detection
It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be 20.00 pg/ml.

Specificity:
The antibodies used in this kit are monoclonal antibodies specific for Mouse BDNF.

Precision:
Intra-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Mouse BDNF were tested in triplicate respectively. The Intra-Assay was found to be <15%
Inter-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Mouse BDNF were tested in triplicate on two plates respectively on two consecutive days. The Inter-Assay was found to be <18%.
The Cumulative Variance % was calculated as CV (%) = SD/mean x 100 [SD=standard deviation]

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from Mouse body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth

- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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SYMBOLS KEY

MTP	Anti-Mouse BDNF Antibody Microtiter Plate (12X8 wells)
STD	Mouse BDNF Standard, lyophilized
BIO CONJ	Biotinylated Anti- Mouse BDNF Detection Antibody
STRP HRP	Streptavidin Horseradish Peroxidase
1X ASY DIL	(1X) Assay Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalogue Number
	Expiration Date
	Storage Temperature