HUMAN GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN GDNF CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND EDTA PLASMA

THIS PROTOCOL OR DATA IS PROVIDED FOR DEMONSTRATION ONLY. ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN GDNF ELISA**
Catalog No.	SK00306-01
Lot No.	
Formulation	96 T
Standard Range	15.6 - 1000 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µL
Sample Type	Cell Culture Supernates, Serum, EDTA Plasma
Specificity	Human GDNF
Calibration	Human GDNF recombinant
Sample Dilution	**Optimal dilutions should be determined by each laboratory for each application**
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	Refer to lot
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human GDNF ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural GDNF from EDTA plasma, serum samples as well as cell culture supernates in a sandwich ELISA format.

This immunoassay contains recombinant GDNF and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural GDNF samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with an antibody specific for GDNF. The capture antibody can bind to the GDNF in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against GDNF is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of GDNF bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
GDNF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against GDNF.	**306-01-01**	**1 plate**
GDNF Standard – refer to lot of recombinant human GDNF in a buffered protein base with preservative; lyophilized.	**306-01-02**	**1 vial**
Detection Antibody Concentrate – refer to lot concentrate of biotinylated antibody against GDNF with preservative; lyophilized.	**306-01-03**	**1 vial**
Positive Control - one vial of recombinant human GDNF; lyophilized.	**306-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µL/vial of 200-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 30 mL of buffered protein based solution with preservative.	**DB108A**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB101**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB68C**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl solution.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for one month. For longer storage up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate ,Dilution Buffer, Antibody Diluent

Solution and HRP diluent Solution should be stored at -20° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Human plasma or serum samples DO NOT need to be diluted.

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

GDNF Standard - Reconstitute the GDNF standard with refer to lot of Dilution Buffer. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (see below). Mix each tube thoroughly before the next transfer. The **1000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	Refer to lot	1000 pg/ml
# 1	250µl of stock	250µl	500 pg/ml
# 2	250µl of 1	250µl	250 pg/ml
# 3	250µl of 2	250µl	125 pg/ml
# 4	250µl of 3	250µl	62.5 pg/ml
# 5	250µl of 4	250µl	31.25 pg/ml
# 6	250µl of 5	250µl	15.625 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to lot of **Antibody Diluent Solution (DB101)** to produce a 10-fold concentrated stock solution. Pipette refer to lot of **Antibody Diluent Solution (DB101)** into a 15 mL centrifuge tube and transfer refer to lot of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of **HRP Diluent Solution (DB68C)** into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution **(protect from light)**.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard dilutions in reverse order of serial dilutions, samples, or positive control per well. Cover with the plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVE

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

STANDARD (pg/mL)	AVERAGE OD450nm (CORRECTED)
Blank	0 (refer to lot)
15.625	0.048
31.25	0.72
62.5	0.103
125	0.228
250	0.533
500	1.107
1000	1.787

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human GDNF	100
Human BDNF	0
Human CNTF	0
Human CTGF	0
Human GRN	0
Human CHGA (19-131)	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate refer to lot on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450 nm within 15 min.