Quantikine® ELISA

Human GDNF Immunoassay

Catalog Number DGD00

For the quantitative determination of human GDNF concentrations in cell culture supernates, cell lysates, tissue lysates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Glial Cell Line-derived Neurotrophic Factor (GDNF) is a neurotrophic factor that has been shown to promote the survival of various neuronal subpopulations in both the central and peripheral nervous systems at different stages of their development. Neuronal subpopulations shown to be affected by GDNF include motor neurons, midbrain dopaminergic neurons, Purkinje cells and sympathetic neurons (1-3).

Native GDNF, a disulfide-linked homodimeric glycoprotein, is a novel member of the TGF-β superfamily. Human GDNF cDNA encodes a 211 amino acid (aa) residue pre-propeptide that is processed to yield a dimeric protein. Mature human GDNF is predicted to contain two 134 aa residue subunits. Mature rat and human GDNF exhibit approximately 93% aa sequence homology and show considerable species cross-reactivity. GDNF is produced by Sertoli cells, type 1 astrocytes, Schwann cells, neurons, pinealocytes, and skeletal muscle cells. GDNF binding to GFR alpha 1 induces the recruitment of Ret, NCAM-1/CD56, various integrins, Syndecan-3, or N-Cadherin. GDNF-based therapies show promise in several neurodegenerative disorders, particularly Parkinson's disease (1,2). Both GDNF and BDNF may contribute to pathological mechanisms involved in unmedicated schizophrenia patients (3).

The Quantikine® Human GDNF Immunoassay is a 4.0 hour solid phase ELISA designed to measure human GDNF levels in cell culture supernates, cell lysates, tissue lysates, serum, and plasma. It contains NS0-expressed recombinant human GDNF and antibodies raised against the recombinant protein. Results obtained for naturally occurring human GDNF showed linear curves that were parallel to the standard curves obtained using the Quantikine® Human GDNF Immunoassay standards. These results indicate that this kit can be used to determine relative mass values for natural human GDNF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human GDNF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any GDNF present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for human GDNF is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, an enzyme-linked streptavidin is added to the wells. After washing away any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and colors develops in proportion to the amount of GDNF bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- 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.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human GDNF Microplate	899452	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human GDNF.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human GDNF Standard	899454	2 vials of recombinant human GDNF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new standard for each assay.
Human GDNF Conjugate	899453	21 mL of a polyclonal antibody specific for human GDNF conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Streptavidin-HRP 1	898926	21 mL of an enzyme solution with preservatives.	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	2 vials of 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- **Use polypropylene tubes** for dilution of standards and samples.
- Human GDNF Controls (optional; R&D Systems®, Catalog # QC253).

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Sample Activation Kit (R&D Systems®, Catalog # DY010).
- Cell Lysis Buffer 3 (R&D Systems®, Catalog # 895366), Lysis Buffer 16 (R&D Systems®, Catalog # 895935) or Lysis Buffer 17 (R&D Systems®, Catalog # 895943).
- Total protein assay.

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- Sample Activation Kit (R&D Systems®, Catalog # DY010).
- RIPA Buffer with protease inhibitors.
- Total protein assay.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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

Cell Lysates/Tissue Lysates - Lysates were prepared as described in the Sample Values section on page 11.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature 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 or heparin 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.

Note: *Citrate plasma has not been validated for use in this assay. Grossly hemolyzed samples are not suitable for use in this assay.*

CELL AND TISSUE LYSATE PRETREATMENT

Use the following procedure for the preparation of cell lysate and tissue lysate samples.

Quantitation of sample protein concentration using a total protein assay is recommended. The suggested range for total cell lysate protein added is 2-100 µg/well.

1. Add 50 µL 1 N HCl to 100 µL sample and mix well.
2. Leave the treated sample to sit on the benchtop for 10 minutes.
3. Add 50 µL 1.2 N NaOH/0.5 HEPES to treated sample and mix well.
4. **Assay immediately.** Discard treated sample after use. Use a fresh treated sample for each assay.
   Note: *Since the samples are pretreated, the concentration read from the standard curve must be multiplied by the dilution factor, 2.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

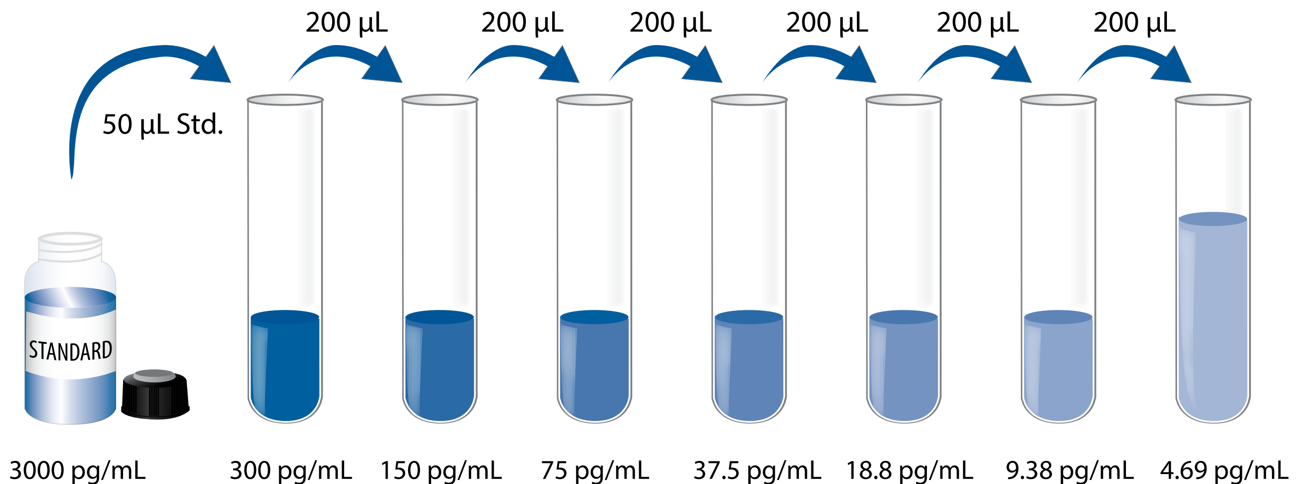
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 10 mL of Calibrator Diluent RD5P to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:5).

Human GDNF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human GDNF Standard with deionized or distilled water. This reconstitution produces a stock solution of 3000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 450 μL of Calibrator Diluent RD5P (diluted 1:5) into the 300 pg/mL tube. Pipette 200 μL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 300 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 pg/mL).



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ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for **2 hours** at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 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.
6. Add 200 µL of Human GDNF Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Streptavidin-HRP 1 to each well. Incubate for 30 minutes at room temperature on the shaker.
9. Repeat the aspiration/wash as in Step 5.
10. Add 200 µL of the Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
11. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Cell and tissue lysates require a pretreatment. See Cell and Tissue Lysate Sample Preparation section.

CALCULATION OF RESULTS

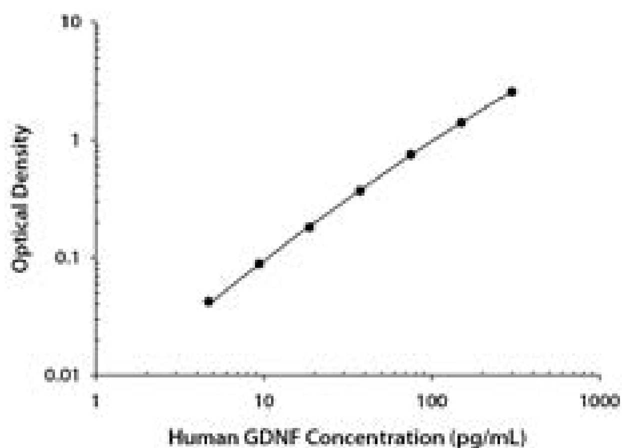
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human GDNF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.010 0.011	0.011	—
4.69	0.053 0.053	0.053	0.042
9.38	0.098 0.102	0.100	0.089
18.8	0.188 0.194	0.191	0.180
37.5	0.379 0.385	0.382	0.371
75	0.757 0.764	0.761	0.750
150	1.419 1.421	1.420	1.409
300	2.545 2.545	2.545	2.534

PRECISION

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

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	30.1	92.2	183	29.7	88.2	178
Standard deviation	0.727	1.38	3.40	1.82	5.16	10.3
CV (%)	2.4	1.5	1.9	6.1	5.8	5.9

RECOVERY

The recovery of human GDNF spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	85-112%
Lysis Buffer* (n=4)	99	93-107%
Serum (n=5)	86	72-99%
EDTA plasma (n=5)	81	65-91%
Heparin (plasma=5)	84	69-95%

*Sample were pretreated prior to assay.

SENSITIVITY

Twenty-three assays were evaluated and the minimum detectable dose (MDD) of human GDNF ranged from 0.160-0.696 pg/mL. The mean MDD was 0.329 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of the standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human GDNF were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Lysis buffer* (n=4)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	99	100	107	109	107
	Range (%)	98-100	98-102	101-114	105-117	103-113
1:4	Average % of Expected	99	101	112	113	112
	Range (%)	97-101	98-105	103-122	104-125	103-121
1:8	Average % of Expected	99	103	118	117	115
	Range (%)	97-102	98-108	104-130	104-132	105-127
1:16	Average % of Expected	96	101	116	120	115
	Range (%)	91-99	95-108	105-128	107-137	106-128

*Samples we pretreated prior to assay.

CALIBRATION

This immunoassay is calibrated against highly purified NS0-derived recombinant human GDNF produced at R&D Systems®.

The NIBSC/non-WHO Human GDNF Reference Material 09/266 (rDNA derived), which was intended as a potency standard, was evaluated in this kit.

The dose response curve of this NIBSC/non-WHO Reference Material parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human GDNF kit to approximate NIBSC/non-WHO 09/266 values, use the equation below.

NIBSC/non-WHO Reference Material (09/266) approximate value (IU/mL) = 0.0012 x Quantikine® Human GDNF value (pg/mL)

Note: *Based on data generated in January 2019.*

SAMPLE VALUES

Serum/Plasma - Thirty samples from apparently healthy volunteers were evaluated for the presence of human GDNF in this assay. No detectable levels were observed. No medical histories were available for the donors used in this study.

Cell Culture Supernates/Cell Lysates*: U-87 MG human glioblastoma/astrocytoma cells were cultured in MEM NEAA Earle's Salts supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate and grown until confluent.

For cell culture supernates, U-87 MG cells along with the cell culture conditioned media supernate were centrifuged at 500 x g for 5 minutes. Aliquots of the cell culture supernates were removed and assayed for human GDNF.

For cell lysates, U-87 MG cells were solubilized in Lysis Buffer 17 and allowed to sit on ice for 30 minutes. Tubes were then centrifuged at 14,000 x g for 5 minutes to remove insoluble material, and the remaining whole cell extract was removed. Whole cell extract protein concentration was quantified using a total protein assay. 100 ug of the cell lysate based on total protein analysis was removed and assayed for human GDNF.

Sample Type	(pg/mL)
Cell culture supernates	44.0
Cell lysates*	8.74

Tissue Lysates* - Brain tissues were rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of RIPA buffer containing protease inhibitors was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Whole tissue extract protein concentration was quantified using a total protein assay. 100 ug of the tissue lysate based on total protein analysis was removed and assayed for human GDNF.

Tissue lysates*	(pg/mL)
Motor cortex	ND
Cerebellum	11.5
Hypothalamus	12.9

ND=Non-detectable

*Lysates were pretreated prior to assay as directed in the Lysate Pretreatment section.

SPECIFICITY

This assay recognizes natural and recombinant human GDNF.

The factors listed below were prepared at ≥ 3 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 3 ng/mL in a mid-range recombinant human GDNF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Artemin
BDNF
CNTF
C-Ret
GFRα-1/GDNF Rα-1
GFRα-2/GDNF Rα-2
GFRα-3/GDNF Rα-3
Neurturin
NGF-β
NT-3
NT-4
Persephin

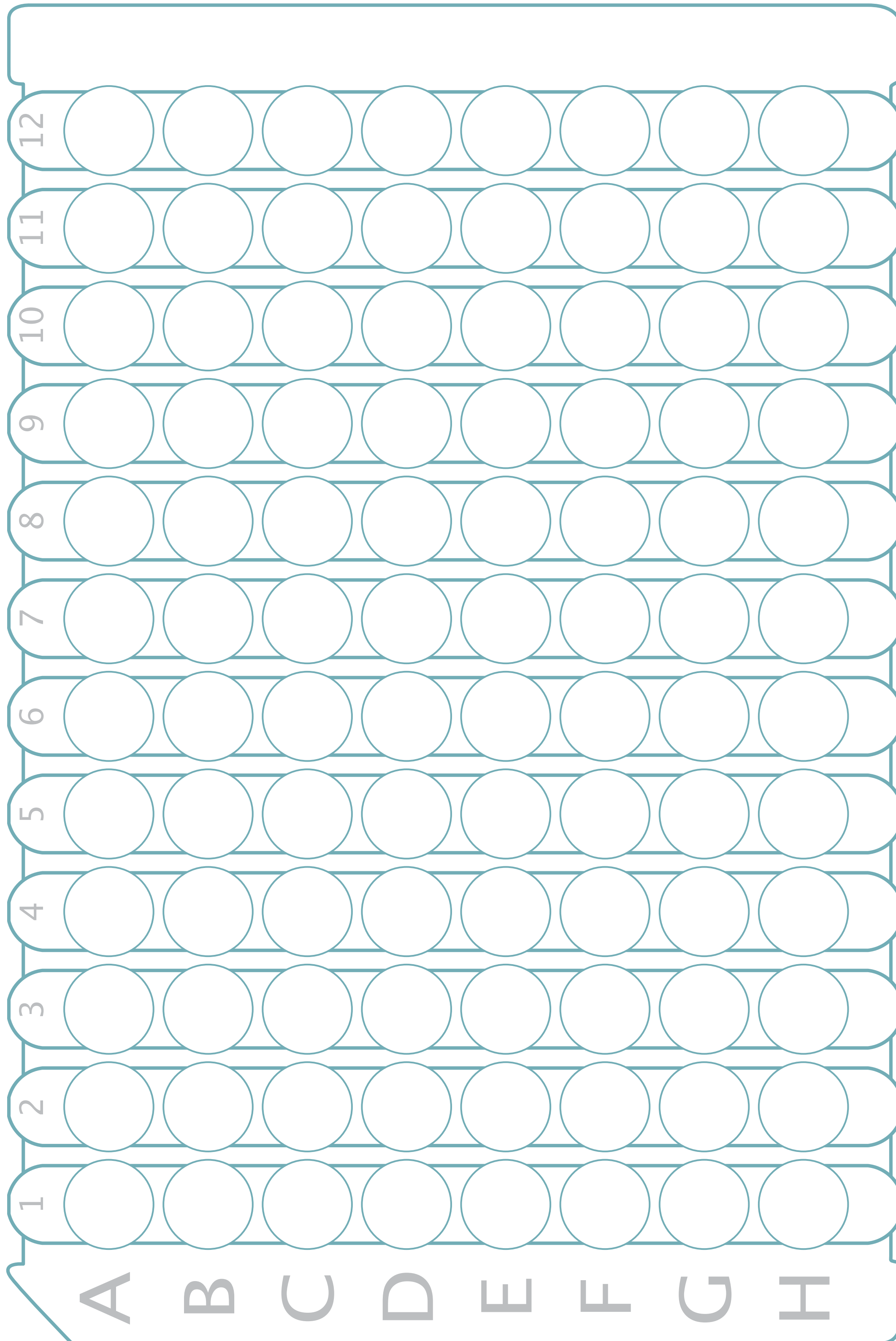
Recombinant rat GDNF does not interfere but does cross-react approximately 39% in this assay.

REFERENCES

1. Rodriquez-Nogales, C. (2016) Maturitas **84**: 25.
2. Torres-Ortega, P.V. (2018) J. Controlled Release **295**: 201.
3. Chu, C.S. (2018) J Chin. Med. Assoc. **81**(6):577.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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