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Datasheet for ABIN625302 **GDNF ELISA Kit**

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Overview

Quantity:	96 tests
Target:	GDNF
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	4-2000 pg/mL
Minimum Detection Limit:	4 pg/mL
Application:	ELISA

Product Details

Purpose:	Human GDNF ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin, BDNF, BLC, ENA-78, FGF- 4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL- 12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.
Sensitivity:	< 4 pg/mL
Characteristics:	Strip plates and additional reagents allow for use in multiple experiments

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	 Quantitative protein detection Establishes normal range The best products for confirmation of antibody array data
Components:	 Pre-Coated 96-well Strip Microplate Wash Buffer Stop Solution Assay Diluent(s) Lyophilized Standard Biotinylated Detection Antibody Streptavidin-Conjugated HRP TMB One-Step Substrate
Material not included:	 Distilled or deionized water Precision pipettes to deliver 2 µL to 1 µL volumes Adjustable 1-25 µL pipettes for reagent preparation 100 µL and 1 liter graduated cylinders Tubes to prepare standard and sample dilutions Absorbent paper Microplate reader capable of measuring absorbance at 450nm Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	GDNF
Alternative Name:	GDNF (GDNF Products)
Background:	The Human GDNF (Glial Derived Neurotrophic Factor) ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human GDNF in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human GDNF coated on a 96-well plate. Standards and samples are pipetted into the wells and GDNF present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human GDNF antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of GDNF bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	2668

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Target Details

UniProt:	P39905
Pathways:	RTK Signaling, Synaptic Membrane, Tube Formation, Autophagy, Smooth Muscle Cell Migration

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples2 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual.
	2. Add 100 μ L of standard or sample to each well.
	3. Incubate 2.5 h at RT or O/N at 4 °C.
	4. Add 100 μ L of prepared biotin antibody to each well.
	5. Incubate 1 h at RT.
	6. Add 100 μ L of prepared Streptavidin solution to each well.
	7. Incubate 45 min at RT.
	8. Add 100 μ L of TMB One-Step Substrate Reagent to each well.
	9. Incubate 30 min at RT.
	10. Add 50 μL of Stop Solution to each well.
	11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use.
	2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used
	for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) is used for dilution of culture
	supernantants and urine. Suggested dilution for normal serum/plasma: 2 fold*. *Please note
	that levels of the target protein may vary between different specimens. Optimal dilution factors
	for each sample must be determined by the investigator.
	3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
	4. Preparation of standard: Briefly spin the vial of Item C and then add 400 μL Assay Diluent A
	(for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernates/urine) into Item
	C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 20
	μL GDNF standard (50 ng/mL) from the vial of Item C, into a tube with 480 μL Assay Diluent A
	or 1x Assay Diluent B to prepare a 2,000 pg/mL standard solution. Pipette 400 μ L Assay Diluen
	A or 1x Assay Diluent B into each tube. Use the 2,000 pg/mL standard solution to produce a
	dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay
	Diluent B serves as the zero standard (0 pg/mL). 20 μ L standard + 480 μ L 200 μ L 200 μ L 200 μ
	200 μL 200 μL 200myl 2,000 666.7 222.2 74.07 24.69 8.23 2.74 0 pg/mL pg/mL pg/mL pg/mL
	pg/mL pg/mL pg/mL

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	5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature
	and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or
	distilled water to yield 400 ml of 1x Wash Buffer.
	6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μ L of 1x Assay Diluent B
	into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the
	concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be
	diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.
	7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix
	gently before use. HRP-Streptavidin concentrate should be diluted 400-fold with 1x Assay
	Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add
	25 μL of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent B to prepare a
	final 400 fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day
	use). Mix well.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is
	recommended that all standards and samples be run at least in duplicate.
	2. Add 100 μ L of each standard (see Reagent Preparation step 2) and sample into appropriate
	wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with
	gentle shaking.
	3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with
	Wash Buffer (300 myl) using a multi-channel Pipette 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.
	4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well.
	Incubate for 1 hour at room temperature with gentle shaking.
	5. Discard the solution. Repeat the wash as in step
	6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well.
	Incubate for 45 minutes at room temperature with gentle shaking.
	7. Discard the solution. Repeat the wash as in step
	8. Add 100 μL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30
	minutes at room temperature in the dark with gentle shaking.
	9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and
	subtract the average zero standard optical density. Plot the standard curve on log-log graph
	paper or using Sigma plot software, with standard concentration on the x-axis and absorbance
	on the y-axis. Draw the best-fit straight line through the standard points.

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Application Details	
	Typical Data: These standard curves are for demonstration only. A standard curve must be run
	with each assay. Assay Diluent A Human GDNF concentration (pg/mL) 1 10 100 1000 10000 O
	D =4 50 n m 0.001 0.01 0.1 1 10 Assay Diluent B Human GDNF concentration (pg/mL) 1 10 100
	1000 10000 O D =4 50 n m 0.01 0.1 1 10
	Sensitivity: The minimum detectable dose of GDNF is typically less than 4 pg/mL.
	Recovery: Recovery was determined by spiking various levels human GDNF into human serum,
	plasma and cell culture media. Mean recoveries are as follows: Sample Type Average $\%$
	Recovery Range (%) Serum 102.9 92-114 Plasma 99.25 86-113 Cell culture media 119.8 85-
	140
	Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 77.70
	106.6 102.1 Range (%) 70-90 89-132 92-110 1:4 Average % of Expected 76.53 104.3 101.3
	Range (%) 68-91 88-132 90-109
	Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only
Handling	
Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated
	freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is
	recommended to store at -80°C.
Expiry Date:	6 months
Publications	
Product cited in:	Faroni, Smith, Lu, Reid: "Human Schwann-like cells derived from adipose-derived mesenchymal
	stem cells rapidly de-differentiate in the absence of stimulating medium." in: The European
	journal of neuroscience, Vol. 43, Issue 3, pp. 417-30, (2016) (PubMed).
	Wu, Li, Li, Lin, Yu, Luan, Ma: "Transport of glial cell line-derived neurotrophic factor into
	liposomes across the blood-brain barrier: in vitro and in vivo studies." in: International journal of

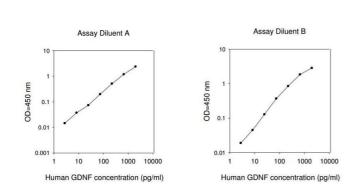
molecular sciences, Vol. 15, Issue 3, pp. 3612-23, (2014) (PubMed).

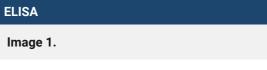
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Kalbermatten, Schaakxs, Kingham, Wiberg: "Neurotrophic activity of human adipose stem cells isolated from deep and superficial layers of abdominal fat." in: **Cell and tissue research**, Vol. 344, Issue 2, pp. 251-60, (2011) (PubMed).

Whone, Kemp, Sun, Wilkins, Scolding et al.: "Human bone marrow mesenchymal stem cells protect catecholaminergic and serotonergic neuronal perikarya and transporter function from oxidative stress by the secretion of glial-derived neurotrophic ..." in: **Brain research**, Vol. 1431, pp. 86-96, (2011) (PubMed).

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