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

SPECIFICITY
The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:
- BDNF
- CNTF
- ß-NGF
- Neurturin
- NT-3
- NT-4

A sample containing 3125 pg/mL of recombinant rat GDNF measured 1084 pg/mL (35% cross-reactivity).

TECHNICAL HINTS & LIMITATIONS
- We recommend the use of R&D Systems™ Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare Reagent Diluent for use in this assay.
- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent is crucial for the optimum performance of the DuoSet® ELISA Development kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.
- It is suggested to start Reagent Diluent optimization for serum and plasma samples by using PBS supplemented with 10-50% animal serum. Do not use buffers with animal serum to reconstitute or dilute the Detection Antibody or Streptavidin-HRP A.
- It is important that the Reagent Diluent selected for reconstitution and dilution of the standard reflects the environment of the samples being measured.
- Avoid microbial contamination of reagents and buffers.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is recommended that all standards and samples be assayed in duplicate.
- The use of PBS from tablets may interfere in this assay.

TROUBLESHOOTING
Note: For more detailed troubleshooting, please visit: www.RnDSystems.com/ELISADevelopment

Poor Standard Curve
- Impure BSA used for Reagent Diluent preparation.
- Improper reconstitution and/or storage of standard.
- Improper dilution of highest standard and standard curve.
- Incomplete washing and/or aspiration of wells.
- Unequal mixing of reagents.

Poor Precision
- Unequal volumes added to wells/pipetting error.
- Incomplete washing and/or aspiration of wells.

Low or No color Development
- Inadequate volume of substrate added to wells.
- Incorrect incubation times or temperatures.
- Impure BSA used for Reagent Diluent preparation.
- Unequal volumes added to wells/pipetting error.
- Incorrect incubation times or temperatures.

INTENDED USE
For the development of sandwich ELISAs to measure natural and recombinant human Glial Cell Line-derived Neurotrophic Factor (GDNF). The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

This kit contains sufficient materials to run ELISAs on at least fifteen 96 well plates, provided the following conditions are met:
- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

This package insert must be read in its entirety before using this product. Refer to the Certificate of Analysis for component concentrations as they may vary. For research use only. Not for use in diagnostic procedures.

FINISHED VOLUME
The finished volume of this assay is typically 150 µL per well.

INTERPRETATION
The following factors will affect the results of this assay.
- Sample preparation
- Reagent preparation
- Technical hints & limitations
- Troubleshooting

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www.RnDSystems.com
1:1 mixture of Color Reagent A (H2O2) and Color Substrate Solution:

1% BSA in PBS, pH 7.2-7.4, 0.2 μm filtered 
Wash Buffer:

0.05% Tween® 20 in PBS, pH 7.2-7.4

The components listed above may be purchased separately:

96 well microplates: (R&D Systems®, Catalog # DY990).

Plate Seals: (R&D Systems®, Catalog # DY992).
PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.2-7.4, 0.2 μm filtered (R&D Systems®, Catalog # DY006).

Wash Buffer: 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems®, Catalog # WA126).

Reagent Diluent: 1% BSA in PBS, pH 7.2-7.4, 0.2 μm filtered (R&D Systems®, Catalog # DY995).

Quality of BSA is critical (see Technical Hints).

PRECAUTIONS

The standard provided with this DuoSet® contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal. Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

The Stop Solution suggested for use with this kit is an acid solution.

The Color Reagent B suggested for use with this kit 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.

CALIBRATION

This DuoSet® is calibrated against a highly purified NS0-expressed recombinant human GDNF produced at R&D Systems®.

REAGENT PREPARATION

Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Streptavidin-HRP A: 1.0 mL of streptavidin conjugated to horseradish-peroxidase. Dilute to the working concentration specified on the vial label using Reagent Diluent.

Mouse Anti-Human GDNF Capture Antibody: Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.

Biotinylated Goat Anti-Human GDNF Detection Antibody: Refer to the lot-specific Certificate of Analysis (C of A) for storage conditions.

Recombinant Human GDNF Standard: Refer to the lot-specific Certificate of Analysis (C of A) for amount supplied. Reconstitute each vial with 0.5 mL of Reagent Diluent. A seven point standard curve using 2-fold serial dilutions in Reagent Diluent is recommended. Prepare 1000 μL of high standard per plate assayed at the concentration indicated on the C of A.

ASSAY PROCEDURE

Determine the optical density of each well immediately, 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.