

# Human neurogranin ELISA Kit Sandwich ELISA Catalog EL-213-14

## INTRODUCTION

Neurogranin, the protein product of NRGN, is a small postsynaptic protein concentrated in the dendritic spines of forebrain neurons, where it acts as a substrate for protein kinase C. In its unphosphorylated state it binds and sequesters calmodulin; when synaptic activity drives calcium influx through NMDA receptors, calmodulin is released, either via phosphorylation of neurogranin or direct calcium-driven dissociation, freeing the calmodulin pool to activate downstream signaling important for synaptic plasticity and memory formation. Because neurogranin expression appears to be influenced by thyroid hormone signaling in the developing brain, changes in this gene's activity have been proposed to contribute to the cognitive effects seen in hypothyroidism. Reflecting its concentration in synaptic terminals, neurogranin leaks into cerebrospinal fluid as synapses degenerate, and rising CSF levels are used to track progression of neurodegenerative conditions such as Alzheimer disease.

## PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format. The microtiter plate is pre-coated with a capture antibody specific to human neurogranin (NRGN); standards or samples are added to the appropriate wells along with a biotin-conjugated detection antibody that also recognizes human NRGN. Avidin conjugated to horseradish peroxidase (HRP) is then added to each well and allowed to incubate. Upon addition of TMB substrate solution, a colorimetric signal develops exclusively in wells where human NRGN, the biotin-conjugated detection antibody, and the avidin-HRP conjugate are all present. The enzymatic reaction is stopped by the addition of a sulphuric acid stop solution, and the resulting color change is measured spectrophotometrically at 450 nm ( $\pm 10$  nm). Sample NRGN concentrations are determined by comparing the optical densities of the samples against a standard curve.

## MATERIALS AND STORAGE

Store kit components at  $-20^{\circ}\text{C}$  unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

| Each kit includes:                    | Units               |
|---------------------------------------|---------------------|
| Pre-Coated Microplate                 | 12 strips x 8 wells |
| Standard (Lyophilized)                | 2 vials             |
| Biotinylated Antibody (100 $\times$ ) | 120 $\mu\text{L}$   |
| Streptavidin-HRP (100 $\times$ )      | 120 $\mu\text{L}$   |
| Standard/Sample Diluent Buffer        | 20 mL               |
| Biotinylated Antibody Diluent         | 12 mL               |
| HRP Diluent                           | 12 mL               |
| Wash Buffer (25 $\times$ )            | 20 mL               |
| TMB Substrate Solution                | 10 mL               |
| Stop Reagent                          | 6 mL                |
| Plate Covers                          | 2 pieces            |

*Do not mix or substitute reagents with those from other lots.*

## MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000 $\mu\text{L}$
- Multi-channel pipette calibrated to deliver 50-200 $\mu\text{L}$
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

## SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators

should wear gloves and protective clothing when handling any patient sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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## PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
- 2. Detection Reagent (1X) Preparation: Dilute the biotinylated antibody and streptavidin-HRP concentrates 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively, immediately before use.**
- 3. Preparation of Calibrators: Prepare calibrators by serial dilution from a 10 down to 0.16 (plus a 0 blank), per the source manual.**

| Sol'n ID | Source         | Source Vol (μL) | Cal. Diluent (μL) | Final Vol (μL) | Final Conc. (ng/mL) |
|----------|----------------|-----------------|-------------------|----------------|---------------------|
| Std 1    | Stock Standard | 500             | 500               | 1000           | 10                  |
| Std 2    | Std 1          | 500             | 500               | 1000           | 5                   |
| Std 3    | Std 2          | 500             | 500               | 1000           | 2.5                 |
| Std 4    | Std 3          | 500             | 500               | 1000           | 1.25                |
| Std 5    | Std 4          | 500             | 500               | 1000           | 0.63                |
| Std 6    | Std 5          | 500             | 500               | 1000           | 0.32                |
| Std 7    | Std 6          | 500             | 500               | 1000           | 0.16                |
| Blank    | Std 7          | 500             | 500               | 1000           | 0                   |

## SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

## ASSAY PROCEDURE

1. After the kit is equilibrated at room temperature, add 100 μL of Standard Working Buffer (gradually diluted according to the instructions) or 100 μL of sample to each well, and incubate at 37°C for 80 minutes.
2. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL Biotinylated Antibody Working Solution (1×) to each well, incubate at 37°C for 50 minutes.
3. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL 1× Streptavidin-HRP Working Solution to each well, incubate at 37°C for 50 minutes.
4. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 μL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.
5. Add 50 μL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

## CALCULATIONS AND RESULTS

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each

sample.

3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

#### **PERFORMANCE CHARACTERISTICS**

**Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.**

**Detection Limit: 0.054 ng/mL**

**Recovery: Serum (n = 5): 81-95% (avg 88%); EDTA plasma (n = 5): 94-105% (avg 98%); Heparin plasma (n = 5): 81-95% (avg 88%)**

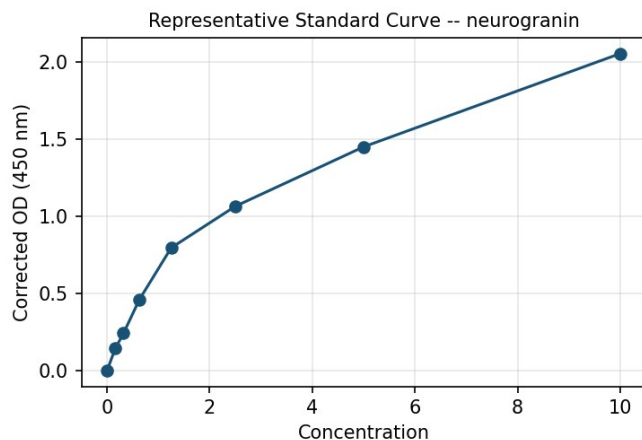
**Specificity: This assay has high sensitivity and excellent specificity for detection of Human NRGN. No significant cross-reactivity or interference between Human NRGN and analogues was observed.**



**This kit employs a sandwich enzyme immunoassay technique to quantify neurogranin (NRGN) in biological samples.**

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### **SAMPLE STANDARD CURVE**



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

### **ORDERING INFORMATION**

Please visit [www.affinityimmuno.com](http://www.affinityimmuno.com) to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.