This kit employs a sandwich enzyme immunoassay technique to quantify Human NRP1 in biological samples.

Human NRP1 ELISA Kit
Sandwich ELISA
Catalog EL-213-14181601

INTRODUCTION

Neuropilin-1 (NRP1) is a large, roughly 130-140 kDa single-pass transmembrane glycoprotein whose extracellular region is built from tandem complement-binding (CUB) domains, coagulation factor V/VIII-like domains, and a MAM domain, followed by a short transmembrane segment and a small cytoplasmic tail. It was first characterized as a receptor that helps guide axons by binding class 3 semaphorins during nervous system development, and it separately functions as a co-receptor that enhances VEGF-A165 signaling through VEGFR2 to drive blood vessel growth. Beyond development and angiogenesis, NRP1 is expressed on endothelial cells, dendritic cells, regulatory T cells, and many tumor cell types, where it also engages latent TGF-beta1 and helps promote regulatory T cell activity, and it acts as a host-cell entry co-factor exploited by SARS-CoV-2. Because of this involvement in vascular growth, immune regulation, and tumor biology, NRP1 is studied both as a cancer therapeutic target and as a marker of endothelial and immune cell activity.

PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format to measure Human NRP1. The microtiter plate wells are pre-coated with a capture antibody specific to Human NRP1. During the assay, standards or samples are added to the appropriate wells together with a biotin-conjugated detection antibody, also specific to Human NRP1, allowing the target analyte to be simultaneously bound by both antibodies. Avidin conjugated to horseradish peroxidase (HRP) is then added to each well and incubated, linking enzymatic activity to the captured complex via the biotin–avidin interaction. Upon addition of TMB substrate solution, a colorimetric signal develops only in wells containing Human NRP1, the biotin-conjugated antibody, and the avidin-HRP conjugate. The enzymatic reaction is stopped by the addition of sulfuric acid stop solution, and absorbance is measured spectrophotometrically at 450 nm (± 10 nm). Human NRP1 concentrations in the samples are determined by comparing sample optical density values against a standard curve.

MATERIALS AND STORAGE

Store kit components at -20°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×)	120 µL
Streptavidin-HRP (100×)	120 µL
Standard/Sample Diluent Buffer	20 mL
Biotinylated Antibody Diluent	12 mL
HRP Diluent	12 mL
Wash Buffer (25×)	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 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- 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 4000 down to 62.5 (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	4000
Std 2	Std 1	500	500	1000	2000
Std 3	Std 2	500	500	1000	1000
Std 4	Std 3	500	500	1000	500
Std 5	Std 4	500	500	1000	250
Std 6	Std 5	500	500	1000	125
Std 7	Std 6	500	500	1000	62.5
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: 26.1 pg/mL

Recovery: Serum (n = 5): 78-90% (avg 84%); EDTA plasma (n = 5): 89-107% (avg 98%); Heparin plasma (n = 5): 87-99% (avg 93%)

Specificity: This assay has high sensitivity and excellent specificity for detection of Human NRP1. No significant cross-reactivity or interference between Human NRP1 and analogues was observed.

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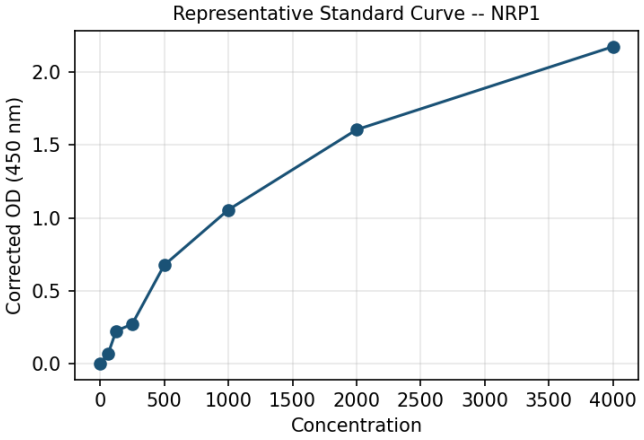
SAMPLE STANDARD CURVE



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* 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.