

total soluble Neuropilin-1

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF TOTAL
SOLUBLE NEUROPILIN-1 IN SERUM, EDTA PLASMA, HEPARIN PLASMA OR
CITRATE PLASMA

CAT. NO. BI-20409 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 180514

This kit was developed and manufactured by:
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CONTENT

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Detailed information on the assay characteristics including the validation data can be found on our website.

www.bmgrp.com

1) INTRODUCTION

Neuropilin-1 (NRP1) is a single-pass transmembrane glycoprotein of 923 amino acids, composed of a large extracellular region, a short transmembrane domain and a short cytoplasmic tail (<https://www.uniprot.org/uniprot/Q14786>). Due to alternative splicing or shedding, the extracellular region can be released into circulation as soluble Neuropilin. NRP1 is an essential cell surface receptor functioning in many key biological processes including the cardiovascular, neuronal, and immune systems (1,2). Multiple ligands bind to the extracellular region of NRP1, like class III semaphorins which have a key role in axonal guidance, or members of the VEGF family of angiogenic cytokines. Ligand-binding to transmembrane NRP1, which has co-receptor function, leads to signaling via receptor proteins containing a PDZ domain. In contrast, ligand-binding to soluble Neuropilin-1 (sNRP1) has antagonistic properties by acting as decoy (1,3).

NRP1 is expressed by a variety of cells and tissues. For instance, the transmembrane protein is expressed by neuronal cells, endothelial cells, vascular smooth muscle cells, cardiomyocytes, multiple tumor cell lines and neoplasms, osteoblasts, naïve T cells or platelets. Expression of soluble Neuropilin-1 is further described in a variety of non-endothelial cells, e.g. in liver hepatocytes and kidney distal and proximal tubules. NRP1 is implicated in a multitude of physiological and pathological settings, e.g. in axon guidance, vascularization, tumor growth or regeneration and repair (4-9). Neuropilin-1 is described to stimulate osteoblast differentiation, to act as potential biomarker for the prediction of heart failure outcome or to play a role in renal fibrogenesis (6, 10,11). As a co-receptor for VEGF, NRP1 is a potential target for cancer therapies (12).

The Neuropilin-1 enzyme immunoassay is a four hour ELISA to quantify human total soluble Neuropilin-1 (sNRP1). The assay is validated for human serum and plasma samples (EDTA, citrate, heparin) (13) (see validation data: www.bmgp.com). To remove potentially bound ligands, samples are pre-treated with guanidine hydrochloride before testing. Recombinant human soluble Neuropilin-1, isoform 2 is used as calibrator.

Areas of Interest

- Oncology
- Osteology
- Nephrology
- Cardiovascular medicine

2) CONTENT OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	Polyclonal sheep anti human Neuropilin-1 antibody precoated microtiter strips in stripholder packed in aluminium bag with desiccant	12 x 8 tests
DILPLATE	Uncoated microtiter plate for sample pre-treatment	12 x 8 wells
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
ASYBUF	Assay buffer, red cap, ready to use	1 x 50 ml
STD	Standards (0; 0.37; 0.75; 1.5; 3; 6; 12 nmol/l), white caps, lyophilised	7 vials
CTRL	Controls A+B, yellow caps, lyophilised, exact concentration see labels	2 vials
AB	Monoclonal mouse anti NRP1 antibody, biotin labelled, green cap, ready to use	1 x 6 ml
CONJ	Conjugate (streptavidin-HRPO), amber cap, ready to use	1 x 18 ml
GuHCl	Guanidin Hydrochloride (GuHCl), clear cap, ready to use	1 x 1.5 ml
SUB	Substrate (TMB solution), blue cap, ready to use	1 x 22 ml
STOP	STOP solution, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL IN THE KIT

- 3 self-adhesive plastic films
- Protocol sheet
- Quality control protocol
- Instruction for use

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision and multichannel pipettes calibrated to deliver 10 µl, 50 µl, 150 µl, 200 µl and disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- Software for calculation of results
- Test-tubes for sample pre-treatment if supplied DILPLATE (pre-dilution plate) is not used

5) REAGENTS AND SAMPLE PREPARATION

All reagents of the kit are stable at 4°C (2-8°C) until expiry date stated on the label of each reagent.

Sample preparation:

Collect venous blood samples by using standardized blood collection tubes. Perform serum or plasma separation by centrifugation according to supplier's instructions of the blood collection devices. The acquired serum or plasma samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower. All samples should undergo only 5 freeze-thaw cycles. Lipemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values. Samples with values above STD7 (12 nmol/l) can be diluted with STD1 (0 nmol/l) before pre-treatment with GuHCl.

Pre-treatment of STD/CTRL/SAMPLE with Guanidin Hydrochloride:

To remove potentially bound ligands, samples are pre-treated with guanidine hydrochloride before testing.

STD/CTRL/SAMPLE have to be pre-treated with GuHCl (Guanidin Hydrochloride) for 30 minutes prior to testing. Pre-treatment is performed in the DILPLATE (uncoated pre-dilution plate) which is supplied in the kit. Alternatively, test-tubes (not supplied in the kit) can be used.

After the 30 minute incubation period of STD/CTRL/SAMPLE with GuHCl in the DIPLATE, ASYBUF (Assay buffer) is added to the wells. Using a multichannel pipette the mixture is transferred from the pre-dilution plate to the PLATE (pre-coated Anti-NRP1 plate) as soon as possible.

For pipetting scheme please see chapter 7) ASSAY PROTOCOL.

For further information on sample stability please visit our website www.bmgrp.com (see Validation Data) or contact our customer service by e-mail export@bmgrp.com or by phone +43/ 1/ 29107-45.

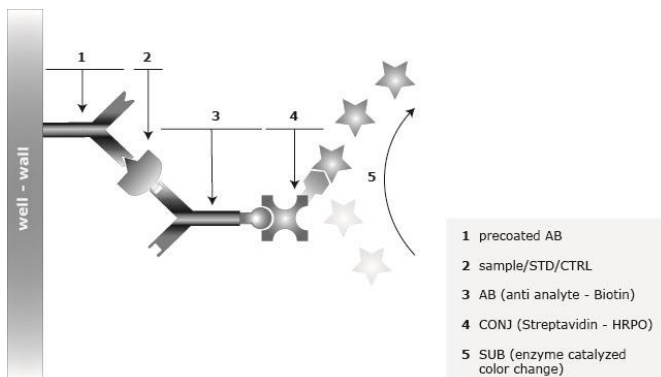
Reagent preparation:

WASHBUF (Wash buffer): Dilute the concentrate 1:20, e.g. 50 ml WASHBUF + 950 ml distilled water. Crystals in the buffer concentrate will dissolve at room temperature. The undiluted WASHBUF is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stable up to one month at 4°C (2-8°C). Only use diluted WASHBUF when performing the assay.

STD (Standards) + CTRL (Controls): Pipette 200 µl of distilled or deionised water into each vial. Leave at room temperature (18-26°C) for 15 min. Vortex gently. The exact concentration is printed on the label. Reconstituted STDs and CTRLs are stable at -25°C or lower until expiry date stated on the label. STDs and CTRLs are stable for 5 freeze-thaw cycles.

6) PRINCIPLE OF THE ASSAY

This kit is a sandwich enzyme immunoassay for the direct determination of total soluble Neuropilin-1 in human serum and plasma samples. STD/CTRL/Sample require pre-treatment with an equal amount of guanidine hydrochloride (GuHCl) to remove potentially bound ligands, followed by pre-dilution with assay buffer. In a next step, pre-treated and diluted STD/CTRL/Sample and detection antibody (mouse monoclonal anti human Neuropilin-1 IgG) are pipetted into the wells of the microtiter strips, which are pre-coated with anti Neuropilin-1 antibody. Neuropilin-1 present in STD/CTRL/Sample binds to the pre-coated antibody in the well and forms a sandwich with the detection antibody. In the washing step all non-specific unbound material is removed. Subsequently, the conjugate (Streptavidin-HRP) is pipetted into the wells and reacts with the detection antibody. After another washing step, the substrate (TMB Tetramethylbenzidine) is pipetted into the wells. The enzyme catalysed colour change of the substrate is directly proportional to the amount of Neuropilin-1. This colour change is detectable with a standard microtiter plate ELISA reader. A dose response curve of the absorbance (optical density, OD at 450 nm) vs. standard concentration is generated, using the values obtained from the standard. The concentration of Neuropilin-1 in the sample is determined directly from the dose response curve.



7) ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-26°C) before use in the assay.

Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.

Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

In pre-dilution plate:

- 1) Pipette 10 µl STD/CTRL/SAMPLE (Standard/Control/Sample) into respective wells.
- 2) Add 10 µl GuHCl (Guanidin Hydrochloride, clear cap) into each well. Swirl gently.
- 3) **Cover tightly and incubate for 30 minutes at room temperature (18-26°C).**
- 4) Add 200 µl ASYBUF (Assay buffer, red cap) into each well. Swirl gently.

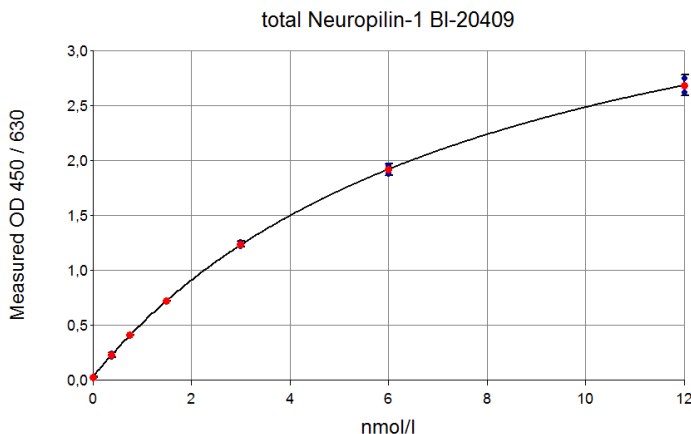
In pre-coated plate:

- 5) Add 50 µl ASYBUF (Assay buffer, red cap) into each well.
- 6) Transfer 50 µl **pre-treated** STD/CTRL/SAMPLE (Standard/Control/Sample) from pre-dilution plate into respective wells. Swirl gently.
For the transfer of pre-treated STD/SAMPLE/CTRL into the coated plate it is recommended to use a multichannel pipette. Transfer should be performed as soon as possible.
- 7) Add 50 µl AB (biotinylated anti NRP-1 antibody, green cap) into each well. Swirl gently.
- 8) **Cover tightly and incubate for 2 hours at room temperature (18-26°C).**
- 9) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, natural cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- 10) Add 150 µl CONJ (Conjugate, amber cap) into each well. Swirl gently.
- 11) **Cover tightly and incubate for 1 hour at room temperature (18-26°C), in the dark.**
- 12) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, natural cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- 13) Add 150 µl SUB (Substrate, blue cap) into each well. Swirl gently.
- 14) **Incubate for 30 min at room temperature (18-26°C) in the dark.**
- 15) Add 50 µl STOP (Stop solution, white cap) into each well. Swirl gently.
- 16) Measure absorbance immediately at 450 nm with reference 630 nm, if available.

8) CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Construct the standard curve from the OD values of the STD. Use commercially available software. Obtain sample concentration from this standard curve. The assay was evaluated with 4PL algorithm. Different curve fitting methods need to be evaluated by the user. Respective dilution factors have to be considered when calculating the final concentration of the sample (e.g samples measuring above STD7).

Typical STD-curve:



The quality control protocol supplied with the kit shows the results of the final release QC for each kit at production date. Data for OD obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an OD of 1.00 or higher is obtained for the standard with the highest concentration and the control value is in range (target range see label).

9) ASSAY CHARACTERISTICS

Method	Sandwich ELISA, HRP/TMB, 12x8-well strips
Sample type	Serum, EDTA plasma, heparin plasma, and citrate plasma
Standard range	0 to 12 nmol/l (0 / 0.37 / 0.75 / 1.5 / 3 / 6 / 12 nmol/l) 7 standards and 2 controls in a human serum matrix.
Conversion factor	soluble Neuropilin-1: 1 ng/ml = 0.014 nmol/l; 1 nmol/l=69.7 ng/ml (MW: 69.7 kDa)
Sample volume	10 µl / well
Incubation time, temp.	30 min / 2 h / 1 h / 30 min, room temperature
Sensitivity	LOD: (0 pmol/l + 3 SD): 0.09 nmol/l; LLOQ: 0.09 nmol/l

Specificity	<p>The assay is optimized to detect total soluble Neuropilin-1 (NRP1) in human plasma and serum. This assay recognizes endogenous and recombinant human soluble Neuropilin-1 (isoform 2 and 3). The total soluble Neuropilin-1 ELISA utilizes a monoclonal anti-human Neuropilin-1 antibody that binds to a linear epitope close to the N-terminus in the CUB 1 domain of the Neuropilin-1 molecule. The polyclonal detection antibody binds to multiple linear epitopes, distributed over the entire Neuropilin-1 molecule. The human sequence homology between NRP1 and NRP2 is low. The amino acid sequence in the respective binding regions of the antibodies show no homology with NRP2. Thus a cross reactivity to NRP2 is not expected.</p>		
Precision	Intra-assay (n=6) ≤ 11%, Inter-assay (n=12) ≤ 10%		
Spike/Recovery (recombinant 1.5 + 6 nmol/l Neuropilin-1)	Average % recovery	1.5 nmol/l	6 nmol/l
	Serum (n=6)	90	92
	EDTA plasma (n=6)	91	93
	Citrate plasma (n=1)	98	108
	Heparin plasma (n=1)	115	97
Dilution linearity of endogenous soluble Neuropilin-1	Average % of expected of dilution:	1+1	1+3
	Serum (n=6):	95	104
	EDTA plasma (n=6):	107	110
	Citrate plasma (n=1):	101	102
	Heparin plasma (n=1):	99	104
Values of apparently healthy individuals	<p>Median serum (n=24) = 2.0 nmol/l Median EDTA plasma (n=24) = 1.7 nmol/l Median heparin plasma (n=24) = 1.9 nmol/l Median citrate plasma (n=24) = 1.7 nmol/l</p> <p>Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.</p>		
Neuropilin-1 in non-human species	<p>The sequence of Neuropilin-1 in mammals is highly conserved. Monkey, pig, rat and mouse show a homology of >90% with human Neuropilin-1. The assay has not been validated for non-human samples, however spike recovery and linearity data in rat and mouse show an acceptable performance (for more information see our validation data file).</p>		

For details on validation data and assay characteristics please visit our website www.bmgrp.com (see Validation Data) or contact our customer service by e-mail export@bmgrp.com or by phone +43/ 1/ 29107-45.

10) PRECISION

Intra-assay: 2 samples of known concentrations were tested 6 times with 1 kit lot by 1 operator.

Inter-assay: 2 samples of known concentrations were tested 12 times with 2 different kit lots on 3 days by 3 different operators.

Intra-assay (n=6)	Sample 1	Sample 2
Mean (nmol/l)	0.8	6.2
SD (nmol/l)	0.1	0.4
CV (%)	11	7

Inter-assay (n=12)	Sample 1	Sample 2
Mean (nmol/l)	0.8	6.2
SD (nmol/l)	0.08	0.36
CV (%)	10	6

11) TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- Substrate solution should remain colourless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

12) PRECAUTIONS

All test components of human source were tested against HIV-Ab, HCV-Ab and HBsAg and were found negative. Nevertheless, they should be handled and disposed of as if they were infectious. Liquid reagents contain $\leq 0.1\%$ Proclin 300 as preservative. Avoid contact with skin and mucous membrane. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin or eyes.

- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Avoid all contact with reagents by using gloves.
- Sulfuric acid is irritating to eyes and skin. Avoid contact with skin and mucous membrane. Irritations are possible – Flush with water if contact occurs!!

13) LITERATURE

1. Neuropilin Functions as an Essential Cell Surface Receptor. HF Guo and CW Vander Kooi, J Biol Chem, 2015; 290(49):29120-29126.
2. Neuropilin signalling in angiogenesis. Koch S, Biochem Soc Trans, 2012; 40(1):20-25.
3. Characterization of neuropilin-1 structural features that confer binding to semaphorin 3A and vascular endothelial growth factor 165. Gu C et al., J Biol Chem, 2002; 277(20): 18069-18076.
4. Multifaceted Role of Neuropilins in the Immune System: Potential Targets for Immunotherapy. Roy S et al., Front Immunol, 2017; 10 (8): 1228.
5. Neuropilin 1 expression in human aortas, coronaries and the main bypass grafts. Alattar M et al., Eur J Cardiothorac Surg, 2014; 46(6): 967-973.
6. Role of Neuropilin-1 in Diabetic Nephropathy. Bondeva T et al., J Clin Med, 2015; 4(6): 1293-1311.
7. Neuropilins: role in signalling, angiogenesis and disease. Zachary I, Chem Immunol Allergy, 2014; 99: 37–70.
8. Neuropilin-1 is upregulated in the adaptive response of prostate tumors to androgen-targeted therapies and is prognostic of metastatic progression and patient mortality. Tse BWC et al., Oncogene, 2017; 36(24): 3417-3427.
9. Dual Function of NRP1 in Axon Guidance and Subcellular Target Recognition in Cerebellum. Telley L et al., Neuron, 2016; 21; 91(6): 1276-1291.
10. Nrp1, a Neuronal Regulator, Enhances DDR2-ERK-Runx2 Cascade in Osteoblast Differentiation via Suppression of DDR2 Degradation. Zhang Y et al., Cell Physiol Biochem, 2015; 36(1): 75-84.
11. Neuropilins: structure, function and role in disease. Pellet-Many C et al., Biochem J, 2008; 411(2): 211-226.
12. Neuropilin-1 as Therapeutic Target for Malignant Melanoma. G Graziani1 and PM Lacal, Front Oncol, 2015; 5: 125.
13. CPMP/ICH/381/95 ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology” including: ICH Q2A “Text on Validation of Analytical Procedures” ICH Q2B “Validation of Analytical Procedures: Methodology”.
14. Neuropilins lock secreted semaphorins onto plexins in a ternary signaling complex. Janssen BJC et al., Nat struct Mol Biol, 2012; 19:1293-1299.

SYMBOLS



Expiry date / Verfallsdatum / Date de péremption / Data di scadenza / Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba expirácie / Doba expirace



Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití



In vitro Diagnostic Medical Device (for in Vitro Diagnostic Use) / In vitro Diagnostikum (zur In-vitro-Diagnostik) / Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro) / Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro) / Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro) / Dispositivo médico para diagnóstico in vitro (Para utilização de diagnóstico "in vitro") / Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) / Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in vitro diagnostisk anvendelse) / Medicinteknisk produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnický materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnický materiál (určeno pro diagnostiku „in vitro“)



Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže



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Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalogové číslo



Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevaars mellem / Förvaras vid / Przechowywać w / Tároljuk között / Skladujte v rozsahu / Skladujte v rozmezí



Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

BI-20409 SOLUBLE NEUROPILIN-1

ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- ☐ Bring all reagents to room temperature (18-26°C).
- ☐ Prepare reagents and samples as instructed.
- ☐ Bring unused and prepared components to the storage temperature mentioned in the package insert.
- ☐ Take microtiter strips out of the aluminium bag and mark positions on the protocol sheet.

TEST PROCEDURE:

IN PRE-DILUTION PLATE:

- ☐ Step 1) Pipette 10 µl STD/CTRL/SAMPLE (Standard/Control/Sample) into respective wells.
- ☐ Step 2) Add 10 µl GuHCl (Guanidin Hydrochloride, clear cap) into each well. Swirl gently.
- ☐ Step 3) **Cover tightly and incubate for 30 minutes at room temperature (18-26°C).**
- ☐ Step 4) Add 200 µl ASYBUF (Assay buffer, red cap) into each well. Swirl gently.

IN PRE-COATED PLATE:

- ☐ Step 5) Add 50 µl ASYBUF (Assay buffer, red cap) into each well.
- ☐ Step 6) Transfer 50 µl **pre-treated** STD/CTRL/SAMPLE (Standard/Control/Sample) from pre-dilution plate into respective wells. Swirl gently.
For the transfer of pre-treated STD/SAMPLE/CTRL into the coated plate it is recommended to use a multichannel pipette. Transfer should be performed as soon as possible.
- ☐ Step 7) Add 50 µl AB (biotinilated anti NRP-1 antibody, green cap) into each well. Swirl gently.
- ☐ **Step 8) Cover tightly and incubate for 2 hours at room temperature (18-26°C).**
- ☐ Step 9) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, natural cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- ☐ Step 10) Add 150 µl CONJ (Conjugate, amber cap) into each well. Swirl gently.
- ☐ **Step 11) Cover tightly and incubate for 1 hour at room temperature (18-26°C), in the dark.**
- ☐ Step 12) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, natural cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- ☐ Step 13) Add 150 µl SUB (Substrate, blue cap) into each well. Swirl gently.
- ☐ **Step 14) Incubate for 30 min at room temperature (18-26°C) in the dark.**
- ☐ Step 15) Add 50 µl STOP (Stop solution, white cap) into each well. Swirl gently.
- ☐ Step 16) Measure absorbance immediately at 450 nm with reference 630 nm, if available.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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