Neuropilin-1 ELISA Kit (Rat) (OKBB00612)

Instruction for Use

For the quantitative measurement of Rat Neuropilin-1 in cell culture supernatants, serum, and plasma (heparin, EDTA).

This product is intended for research use only.

Table of Contents

1. Background

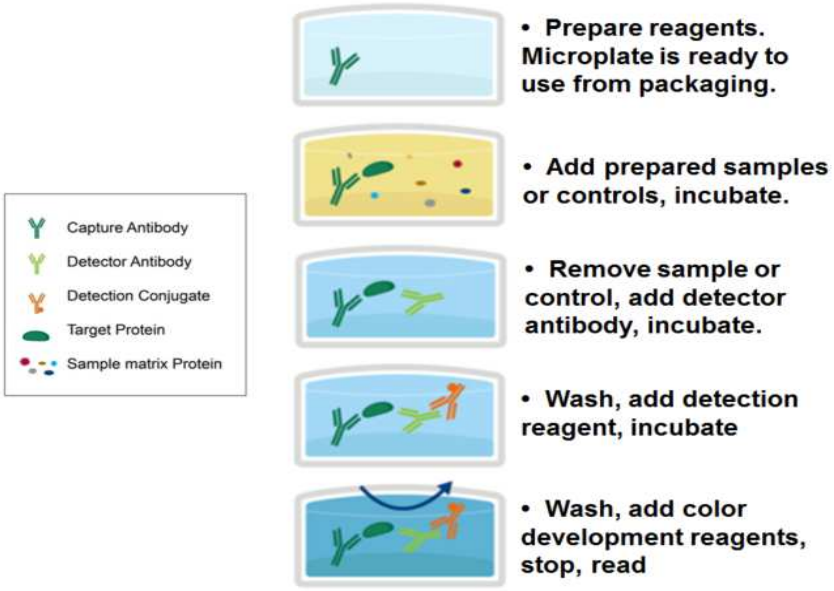
Principle

Aviva Systems Biology Neuropilin-1 ELISA Kit (Rat) (OKBB00612) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. A mouse monoclonal antibody specific for Neuropilin-1 has been pre-coated onto 96-wellplate (12 x 8 Well Strips). Standards (NSO; F22-D854) and test samples are added to the wells and incubated. After washing, a biotinylated polyclonal goat detector antibody specific for Neuropilin-1 is added, incubated and followed by washing. Avidin-Biotin-Peroxidase Complex is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is visualized through the addition of TMB substrate which is catalyzed by HRP to produce a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample Rat Neuropilin-1 captured in well.

Background

NRP1 (Neuropilin 1) also known as NP1, NRP, BDCA4 or VEGF165R, is a membrane-bound coreceptor to a tyrosine kinase receptor for both vascular endothelial growth factor (VEGF) and semaphorin family members. NRP1 plays versatile roles in angiogenesis, axon guidance, cell survival, migration, and invasion. By somatic cell hybrid analysis, the NRP1 gene was mapped to chromosome 10. NRP1 bounds PGF1 with lower affinity. NRP1-mediated interactions are a necessary element in the initiation of the primary immune response and offer another example, like that of agrin, of a molecule shared by neurologic and immunologic synapses. After T-cell contact with DC, T-cell NRP1 colocalized with CD3 in the immunologic synapse and, sometimes, also at the opposite pole of the T cell. Soluble NRP1 interacts in a homophilic fashion with NRP1 on both DC and T cells, and this binding can be inhibited by blocking antibodies to NRP1. Furthermore, selective NRP1 inhibition in this model suppressed neovascular formation substantially.

2. Assay Summary



3. Precautions

• Read instructions fully prior to beginning use of the assay kit.
• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

4. Storage and Stability

• Upon receipt store kit at 4°C for 6 months or -20°C for 12 months. Avoid multiple freeze/thaw cycles.

5. Kit Components

• The following reagents are the provided contents of the kit.

Description	Quantity
96-Well plate Pre-coated with Anti-Rat Neuropilin-1 Antibody	1 (12 x 8 Well Strip)
Lyophilized Recombinant Rat Neuropilin-1 Standard	50 ng/tube × 2
100X Biotinylated Anti-Rat Neuropilin-1 Antibody	130 µL
100X Avidin-Biotin-Peroxidase Complex (ABC)	130 µL
Sample Diluent Buffer	30 mL
Antibody Diluent Buffer	12 mL
ABC Diluent Buffer	12 mL
TMB Color Developing Agent	10 mL
TMB Stop Solution	10 mL
10X Wash Buffer	30 mL

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.

7. Technical Application Tips

• Do not mix or substitute components from other kits.
• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
• Inspect all reagents prior to use. Components should contain no particulates or cloudiness and should be colorless.
• Prior to using the kit, briefly spin component tubes to collect all reagent at the bottom.
• Replicate wells are recommended for standards and samples.
• Cover microplate while incubating to prevent evaporation.
• Do not allow the microplate wells dry at any point during the assay procedure.
• Do not reuse tips or tube to prevent cross contamination.
• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
• Completely remove of all liquids when washing to prevent cross contamination.
• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
• Equilibrate all materials to ambient room temperature prior to use (standards exception).
• For optimal results in inter- intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.
• Pipetting less than 1 µL is not recommended for optimal assay accuracy.
• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
• Samples containing precipitates or fibrin strands or which are hemolytic of lipemic might cause inaccurate results due to interfering factors.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

8.1 1X Biotinylated Anti-Rat Neuropilin-1 Antibody

8.1.1 Prepare the **1X Biotinylated Anti-Rat Neuropilin-1 Antibody** immediately prior to use by diluting the **100X Biotinylated Anti-Rat Neuropilin-1 Antibody** 1:100 with Antibody Diluent Buffer.
8.1.2 For each well to be used in the experiment prepare 120 µL by adding 1.2 µL of **100X Biotinylated Anti-Rat Neuropilin-1 Antibody** to 118.8 µL Antibody Diluent Buffer.
8.1.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.2 1X Avidin-Biotin-Peroxidase Complex (ABC)

8.2.1 Prepare the **1X Avidin-Biotin-Peroxidase Complex (ABC)** immediately prior to use by diluting the **100X Avidin-Biotin-Peroxidase Complex (ABC)** 1:100 with **ABC Dilution Buffer**.
8.2.2 For each well to be used in the experiment prepare 120 µL, by adding 1.2 µL of **100X Avidin-Biotin-Peroxidase Complex (ABC)** to 118.8 µL **ABC Dilution Buffer**.
8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.3 Neuropilin-1 Assay Standards

8.3.1 Prepare the Neuropilin-1 standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.3.2 Reconstitute one of the provided 10 ng **Lyophilized Recombinant Rat Neuropilin-1 Standard**. Use one for each experiment. Prepare a stock 10,000 pg/mL **Rat Neuropilin-1 Standard** by reconstituting one tube of **Lyophilized Recombinant Rat Neuropilin-1 Standard** as follows:

8.3.2.1 Gently spin or tap the vial to collect all material at the bottom.

8.3.2.2 Add 1 mL of **Sample Diluent Buffer** to the vial.

8.3.2.3 Seal then mix gently and thoroughly.

8.3.2.4 Leave the vial to sit at ambient temperature for 10 minutes.

8.3.3 Prepare a set of seven serially diluted standards as follows:

8.3.3.1 Label tubes with numbers 2 – 8.

8.3.3.2 Add 300 µL of **Sample Diluent Buffer** to Tube #'s 2 – 8.

8.3.3.3 Use the reconstituted 50 ng/mL Rat Neuropilin-1 as **Standard #1**.

8.3.3.4 Prepare **Standard #2** by adding 300 µL of **50 ng/mL Standard #1** from Tube #1 to Tube #2. Mix gently and thoroughly.

8.3.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.3.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.3.3.7 Tube #8 is a blank standard (only **Sample Diluent Buffer**), which should be included with every experiment.

Standard Number (Tube)	Sample To Dilute	Volume Standard (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
Tube #1	50 ng/mL of Rat Neuropilin-1 Standard	NA	NA	1,000	50 ng/mL
Tube #2	50 ng/mL	300	300	600	25 ng/mL
Tube #3	25 ng/mL	300	300	600	12.5 ng/mL
Tube #4	12.5 ng/mL	300	300	600	6.25 ng/mL
Tube #5	6.25 ng/mL	300	300	600	3.12 ng/mL
Tube #6	3.12 ng/mL	300	300	600	1.56 ng/mL
Tube #7	1.56 ng/mL	300	300	600	0.78 ng/mL
Tube #8	NA	0	300	300	0.0 (Blank)

8.4 1X Wash Buffer

8.4.1 Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.
8.4.2 Add the entire 30 mL contents of the **10X Wash Buffer** bottle to the water.
8.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.
8.4.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- Clear samples by centrifugation as follows:

- **Cell culture supernatant** - Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
- **Serum** - Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
- **Plasma** - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1,500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

9.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range.

- Prepare 150 µL sample for each replicate to be assayed.
- Dilute samples with Sample Diluent Buffer.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Refer to the following table for recommended sample dilution guidelines based on the dynamic range of this kit:

Estimated Sample Target Concentration		Dilution Level	Sample Volume For Two Replicates	Sample Diluent Buffer For Two Replicates
High Concentration	500-5,000 ng/mL	1:100	3 µL	297 µL
Medium Concentration	50-500 ng/mL	1:10	30 µL	270 µL
Low Concentration	0.78-50 ng/mL	1:2	150 µL	150 µL
Very Low Concentration	≤0.78 ng/mL	1:2 or No Dilution	-	-

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.
• To control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

10.1 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.
10.2 Cover the plate with the well plate lid and incubate for 90 minutes.
10.3 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
10.5 Add 100 µL of prepared **1X Biotinylated Anti-Rat Neuropilin-1 Antibody** to each well.
10.6 Cover with the well-plate lid and Incubate for 60 minutes.
10.7 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
10.8 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
10.9 Wash plate 3 times with **1X Wash Buffer** as follows:
10.9.1 Add 300 µL of **1X Wash Buffer** to each assay well.
10.9.2 Incubate for 1 minute.
10.9.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
10.9.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
10.9.5 Repeat steps 10.9.1 through 10.9.4 **two** more times
10.10 Add 100 µL of prepared **1X Avidin-Biotin-Peroxidase Complex (ABC)** into each well and incubate for 30 minutes.
10.11 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
10.12 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
10.13 Wash plate 5 times with **1X Wash Buffer** as follows:
10.13.1 Add 300 µL of **1X Wash Buffer** to each assay well.
10.13.2 Incubate for 1 minute.
10.13.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
10.13.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
10.13.5 Repeat steps 10.13.1 through 10.13.4 **four** more times.
10.14 Add 90 µL of **TMB Color Developing Agent** to each well and incubate in the dark for 25 - 30 minutes. (NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)
10.15 Add 100 µL of **TMP Stop Solution** to each well. Well color should change to yellow immediately.
10.16 Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.15.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The Rat Neuropilin-1 concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

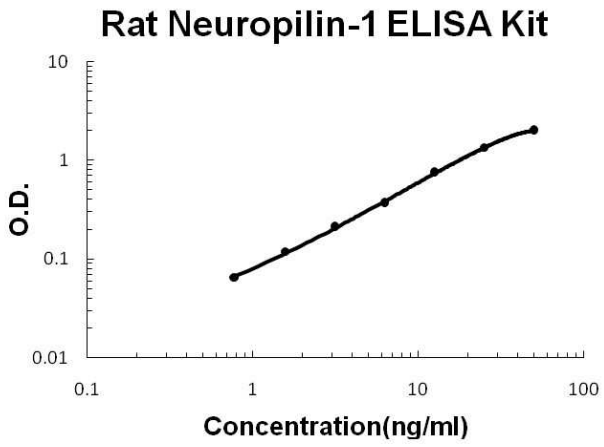
Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1 **Typical absorbance values**. Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD_{450}.

Standard Number	8	7	6	5	4	3	2	1
Concentration (pg/mL)	0	0.78	1.56	3	6	13	25	50
OD_{450}	0.025	0.065	0.119	0.21	0.369	0.749	1.355	2.007

12.2 **Typical standard curve**. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



12.3 General Specifications

General Specificiations	
Range	0.78 ng/mL-50 ng/mL
Sensitivity	< 20 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Natural and recombinant Human Neuropilin-1 UniProt ID: Q9QWJ9
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins
Recovery	-
Linearity	-

12.4 Reproducibility

	Intra-Assay			Inter-Assay		
Sample ID	1	2	3	1	2	3
n =	16	16	16	24	24	24
Mean Measured Concentration (pg/mL)	8.3	21	30	8.6	24	35
Standard Deviation (pg/mL)	0.315	0.987	1.62	0.421	1.32	2.38
Consistency (%CV)	3.8	4.7	5.4	4.9	5.5	6.8

13. Technical Resources

13.1 References

13.1.1 Dzionek, A., Fuchs, A., Schmidt, P., Cremer, S., Zysk, M., Miltenyi, S., Buck, D. W., Schmitz, J. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J. Immun. 165: 6037-6046, 2,000.

13.1.2 Fujisawa, H., Ohtsuki, T., Takagi, S., Tsuji, T. An aberrant retinal pathway and visual centers in Xenopus tadpoles share a common cell surface molecule, A5 antigen. Dev. Biol. 135: 231-240, 1989.

13.1.3 Rossignol, M., Gagnon, M. L., Klagsbrun, M. Genomic organization of human neuropilin-1 and neuropilin-2 genes: identification and distribution of splice variants and soluble isoforms. Genomics 70: 211-222, 2,000.

13.2 Technical Support

USA

Aviva Systems Biology, Corp.
5754 Pacific Center Blvd, Suite 201
San Diego, CA 92121

Phone: 858-552-6979
Toll Free: 888-880-0001
Fax: 858-552-6975

Technical support: techsupport@avivasysbio.com

China

Beijing AVIVA Systems Biology
6th Floor, B Building, Kaichi Tower
#A-2 Jinfu Road.
Daxing Industrial Development Zone
Beijing, 102600, CHINA

Phone: (86)10-60214720
Fax: (86)10-60214722
E-mail: support@avivasysbio.com.cn

中国地址：北京大兴工业开发区金辅路甲 2 号凯驰大厦 B 座 6 层 (102600)

电话: 010-60214720/21

传真: 010-60214722

产品售前咨询及销售: sales@avivasysbio.com.cn

售后及技术支持: support@avivasysbio.com.cn