

Total soluble Neuropilin-1 ELISA, BI-20409

Protocol for the measurement of total soluble Neuropilin-1 in non-human Samples (mouse, rat, pig, primates)

The Biomedica ELISA is fully validated for the measurement of human total soluble Neuropilin-1 in serum, EDTA-plasma, heparin plasma, and citrate plasma.

The ELISA has not been fully validated for the measurement total soluble Neuropilin-1 in non-human samples but can be used for different animal models.

The sequence of Neuropilin-1 in mammals is highly conserved.

Table: Sequence comparison of NRP-1 protein in mouse, human, and rat

P97333 NRP1_MOUSE	1	MERGLPLLCAVLALALAGAFNRSDKCGGTIKIENPGYLTPSGYFHSYHPSEKCEWLIQA	60
O14786 NRP1_HUMAN	1	MERGLPLLCAVLALVLPAGAFNRNDKCGDTIKIESPGYLTPSGYFHSYHPSEKCEWLIQA	60
Q9QWJ9 NRP1_RAT	1	MERGLPLLCAVLALALAGAFNRSDKCGGTIKIENPGYLTPSGYFHSYHPSEKCEWLIQA	60
P97333 NRP1_MOUSE	61	PEFYQRIMINFNPHFDELRDCKYDYVEVDIGENEGRLWGKFCGKIAPSPVSSGGPFLF	120
O14786 NRP1_HUMAN	61	PDFYQRIMINFNPHFDELRDCKYDYVEVFDGENENHFRGKFCGKIAPPVSSGGPFLF	120
Q9QWJ9 NRP1_RAT	61	PEFYQRIMINFNPHFDELRDCKYDYVEVDIGENEGRLWGKFCGKIAPSPVSSGGPFLF	120
P97333 NRP1_MOUSE	121	IKFVSDYETHGAGFSIRYEIFKRGPECSQNYTAPTGVKISPGFPEKYPNSELECTYIIFAP	180
O14786 NRP1_HUMAN	121	IKFVSDYETHGAGFSIRYEIFKRGPECSQNYTTPSGVTKISPGFPEKYPNSELECTYIVFVP	180
Q9QWJ9 NRP1_RAT	121	IKFVSDYETHGAGFSIRYEIFKRGPECSQNYTAPTGVKISPGFPEKYPNSELECTYIIFAP	180
P97333 NRP1_MOUSE	181	KMSEIILEFESFDLEQDSNPPGGMFCRYDRLEIWDGFPFVGPVPHIGRVCGQKTPGRIRSSS	240
O14786 NRP1_HUMAN	181	KMSEIILEFESFDLEQDSNPPGGMFCRYDRLEIWDGFPDVGPHIGRVCGQKTPGRIRSSS	240
Q9QWJ9 NRP1_RAT	181	KMSEIILEFESFDLEQDSNPPGGVFCRYDRLEIWDGFPFVGPVPHIGRVCGQKTPGRIRSSS	240
P97333 NRP1_MOUSE	241	GLSMVFYTDSAIAKEGFSANYSVLQSSISEDFKMEALGMESGEIHSQDITASSQYGTN	300
O14786 NRP1_HUMAN	241	GLSMVFYTDSAIAKEGFSANYSVLQSSVSEDFKMEALGMESGEIHSQDITASSQYGTN	300
Q9QWJ9 NRP1_RAT	241	GLSMVFYTDSAIAKEGFSANYSVLQSSISEDFKMEALGMESGEIHSQDITASSQYGTN	300
P97333 NRP1_MOUSE	301	WSVERSLNYPENGWTPGEDSYKEWIQVDLGLLRFVIAVGTQQAISKETKKKYVVKTYRV	360
O14786 NRP1_HUMAN	301	WSAERSLNYPENGWTPGEDSYREWIQVDLGLLRFVIAVGTQQAISKETKKKYVVKTYKI	360
Q9QWJ9 NRP1_RAT	301	WSVERSLNYPENGWTPGEDSYREWIQVDLGLLRFVIAVGTQQAISKETKKKYVVKTYRV	360
P97333 NRP1_MOUSE	361	DISSNGEDWISLKEGNKALIFQGNINPTDVLGVFVSKPLITRFVRIKPVSWETGISMRFE	420
O14786 NRP1_HUMAN	361	DVSSNGEDWITIKEGNKVPLFQGNINPTDVLVAVFVKPLITRFVRIKIPATWETGISMRFE	420
Q9QWJ9 NRP1_RAT	361	DISSNGEDWITLKEGNKALIFQGNINPTDVLGVFVSKPLITRFVRIKIPASWETGISMRFE	420
P97333 NRP1_MOUSE	421	VYGCKITIDYPCSGMLGMVSLISDSQITASNQADRNWMPENIRLVTSRGWALPSPHPFY	480
O14786 NRP1_HUMAN	421	VYGCKITIDYPCSGMLGMVSLISDSQITSSNQGDRNWMENIRLVTSRSGWALFPAPHSY	480
Q9QWJ9 NRP1_RAT	421	VYGCKITIDYPCSGMLGMVSLISDSQITASNQGDRNWMENIRLVTSRGWALPSPHPFY	480
P97333 NRP1_MOUSE	481	TNEWLQVDLGDDEKIVRGVVIQGGKHRENKVFMRKFKIAYSNNGSDWKTIMDDSKRKAQSF	540
O14786 NRP1_HUMAN	481	INEWLQDLGEEKIVRGVVIQGGKHRENKVFMRKFKIAYSNNGSDWKMIMDDSKRKAQSF	540
Q9QWJ9 NRP1_RAT	481	INEWLQVDLGDDEKIVRGVVIQGGKHRENKVFMRKFKIAYSNNGSDWKMIMDDSKRKAQSF	540
P97333 NRP1_MOUSE	541	EGNNYDTPELRITFPLSTRFIRIYPERATHSGLGLRMELLGCEVEAPTAGTTPNGNV	600
O14786 NRP1_HUMAN	541	EGNNYDTPELRITFPALSTRFIRIYPERATHGGLRMELLGCEVEAPTAGTTPNGNLV	600
Q9QWJ9 NRP1_RAT	541	EGNNYDTPELRAFTEPLSTRFIRIYPERATHSGLGLRMELLGCEVEVPTAGTTPNGNV	600
P97333 NRP1_MOUSE	601	DECDDQANCHSGTGDDFQLTGGTIVLATEKPTIIDSTIQSEFPYGFNCFGWGSHKTF	660
O14786 NRP1_HUMAN	601	DECDDQANCHSGTGDDFQLTGGTIVLATEKPTVIDSTIQSEFPYGFNCFGWGSHKTF	660
Q9QWJ9 NRP1_RAT	601	DECDDQANCHSGTGDDFQLTGGTIVLATEKPTIIDSTIQSEFPYGFNCFGWGSHKTF	660
P97333 NRP1_MOUSE	661	CHWEHDSHAQLRWVSLTSGTGPIDQHTGDGNFIYSQADENQKQKVARLVSPVYSSSAH	720
O14786 NRP1_HUMAN	661	CHWEHDNHVQLKWSVLTSGTGPIDQHTGDGNFIYSQADENQKQKVARLVSPVYSSNSAH	720
Q9QWJ9 NRP1_RAT	661	CHWEHDSHAQLRWVSLTSGTGPIDQHTGDGNFIYSQADENQKQKVARLVSPVYSSSAH	720
P97333 NRP1_MOUSE	721	CMTFWYHMSGSHVGTLRVKLRYQKPEEYDQLVMMVVGQGDHWKEGRVLLHKSCLKYQVI	780
O14786 NRP1_HUMAN	721	CMTFWYHMSGSHVGTLRVKLRYQKPEEYDQLVMAIGHQGDHWKEGRVLLHKSCLKYQVI	780
Q9QWJ9 NRP1_RAT	721	CMTFWYHMSGSHVGTLRVKLHYQKPEEYDQLVMMVVGQGDHWKEGRVLLHKSCLKYQVI	780
P97333 NRP1_MOUSE	781	FEGETGKGNLGGIAVDDISINNHISQEDCAKPTDLDRKKNTEIKIDETGSPGYEGEGED	840
O14786 NRP1_HUMAN	781	FEGETGKGNLGGIAVDDISINNHISQEDCAKPADLDRKKNPEIKIDETGSPGYEGEGED	840
Q9QWJ9 NRP1_RAT	781	FEGETGKGNLGGIAVDDISINNHISQEDCAKPTDLDRKKNTEIKIDETGSPGYE-EGGD	839
P97333 NRP1_MOUSE	841	KNISRKPGNVLKTLDLPILITIIAMSAIGVLLGAVCGVVLYCACWHNGMSERNLSALENYN	900
O14786 NRP1_HUMAN	841	KNISRKPGNVLKTLDLPILITIIAMSAIGVLLGAVCGVVLYCACWHNGMSERNLSALENYN	900
Q9QWJ9 NRP1_RAT	840	KNISRKPGNVLKTLDLPILITIIAMSAIGVLLGAVCGVVLYCACWHNGMSERNLSALENYN	899
P97333 NRP1_MOUSE	901	FELVDGVKLLKDKLNQPSNYSEA	923
O14786 NRP1_HUMAN	901	FELVDGVKLLKDKLNQSTYSEA	923
Q9QWJ9 NRP1_RAT	900	FELVDGVKLLKDKLNQPSNYSEA	922

The listed species below also show a homology of >90% with human Neuropilin-1:

- Sus scrofa (Pig)
- Macaca mulatta (Rhesus macaque)
- Pan paniscus (Pygmy chimpanzee) (Bonobo)
- Gorilla gorilla gorilla (Western lowland gorilla)
- Pongo abelii (Sumatran orangutan) (Pongo pygmaeus abelii)
- Chlorocebus sabaeus (Green monkey) (Cercopithecus sabaeus)
- Aotus nancymaae (Ma's night monkey)
- Neovison vison (American mink) (Mustela vison)
- Callithrix jacchus (White-tufted-ear marmoset)

Suggested protocol for the measurement of human Neuropilin-1 in non-human samples

Follow standard 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 (biotinilated 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.

If required, dilute samples 1+1 with STD1 (provided in the kit) prior to GuHCl treatment.

Performance check:

3 mouse serum samples, 3 rat serum samples and 2 pig serum samples (all sera derived from apparently healthy animals) were tested undiluted and spiked by adding STD7 (final concentration 6 nmol/l of human recombinant Neuropilin-1) directly to all samples (ratio 1+1). The spiked sera were diluted 1+1 and 1+3 with STD1 (containing 0 nmol/l NRP1, supplied in the kit). Sample concentrations were calculated by using STD1-STD7.

- Endogenous Neuropilin-1 was undetectable in tested samples thus no test on specificity (competition) was carried out.
- Animal sera can be spiked with recombinant human NRP1 (final concentration 6 nmol/l) – the average recovery is 90%.
- Spiked animal samples can be diluted 1+1 and 1+3 with STD1 (0 nmol/l) – the average recovery is 111% and 119%.

Table: calculation of animal sample concentrations, and spike recovery

Sample matrix	ID	Ref	NRP1 +6 nmol/l	S/R [%]	dil 1+1	dil 1+3	R [%] 1+1	R [%] 1+3	
mouse serum	M1	0	4.9	81	2.6	1.5	108	121	
mouse serum	M2	0	5.7	95	3.6	1.7	126	123	
mouse serum	M3	0	5.0	84	2.7	1.4	107	112	
rat serum	R1	0	5.0	84	3.2	1.4	129	112	
rat serum	R2	0	5.5	92	2.9	1.6	107	119	
rat serum	R3	0	5.5	91	2.9	1.7	105	127	
pig serum	P1	0	5.3	88	2.8	1.4	106	102	
pig serum	P2	0	6.3	106	3.1	1.7	98	107	
				Mean R [%]	90			111	119

Graph: Neuropilin-1 standard curve (STD1-7) and dilution curves of animal sera spiked with human NRP1 diluted with STD1 (0 nmol/l NRP1)

