BIOMEDICA

Total soluble Neuropilin-1 ELISA, BI-20409

Protocol for the measurement of human total soluble Neuropilin-1 in urine samples

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 urine samples but can be used for this sample matrix.

Performance check:

Urine samples (n=6) from apparently healthy subjects and from patients with kidney disease were assayed with the total soluble Neuropilin-1 ELISA following the standard protocol (including GuHCl treatment) using undiluted urine. Sample concentrations were calculated by using STD1-STD7.

- Endogenous Neuropilin-1 was undetectable in tested samples (cohorts from apparently healthy and kidney disease) thus no test on specificity (competition) was carried out.

- Urine samples can be spiked with recombinant human Neuropilin-1. The average recovery of 6 human urine samples is 105%.

- Urine samples spiked with recombinant human Neuropilin-1 can be diluted 1+1 with STD1 (0 nmol/l) that is supplied in the kit. The average recovery of 6 human urine samples is 94%.

RECOVERY

Recovery was assessed by adding STD7 (supplied in the kit, final concentration 6 nmol/l human recombinant Neuropilin-1) directly to 6 different human urine samples (ratio 1+1).

Data showing spike/recovery of human urine samples:

	Neuropilin-1 [nmol/l]		
Sample ID	**Reference**	**+ 6 nmol/l**	**S/R [%]**
#U1	0.1	6.1	100
#U2	0	6.3	105
#U3	0	6.5	108
#U4	0	6.3	105
#U5	0	6.5	108
#U6	0	6.3	105
		Mean R [%]	**105**

LINEARITY

Dilution linearity was assessed by diluting urine samples spiked with 6 nmol/l recombinant Neuropilin-1 with STD1 (0 nmol/l, supplied in the kit).

Data showing the dilution of recombinant Neuropilin-1 in urine samples:

Sample ID	Neuropilin-1 [nmol/l]		Dil R [%]
	Reference	Dil 1+1	
#U1	6.1	2.6	87
#U2	6.3	2.8	89
#U3	6.5	3.2	98
#U4	6.3	3.0	96
#U5	6.5	3.2	100
#U6	6.3	3.0	94
		Mean R [%]	**94**

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

Follow standard protocol as indicated in the package insert:

In pre-dilution plate:
*Use **10 µl urine sample for GuHCl treatment**.*

Continue with protocol as indicated in the instructions for use.

In pre-coated plate:
*Transfer **50 µl pre-treated urine sample** from pre-dilution plate into respective wells. Swirl gently.*
For the transfer into the coated plate it is recommended to use a multichannel pipette.
Transfer should be performed as soon as possible.

Continue with protocol as indicated in the instructions for use.

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