Angiopoietin-2 Mouse/Rat ELISA

for the quantitative determination of mouse or rat Angiopoietin-2 in serum or plasma
Cat. No. BI-ANG2MR . 12 x 8 tests

FOR RESEARCH USE ONLY

CONTENTS

ASSAY CHARACTERISTICS Summary

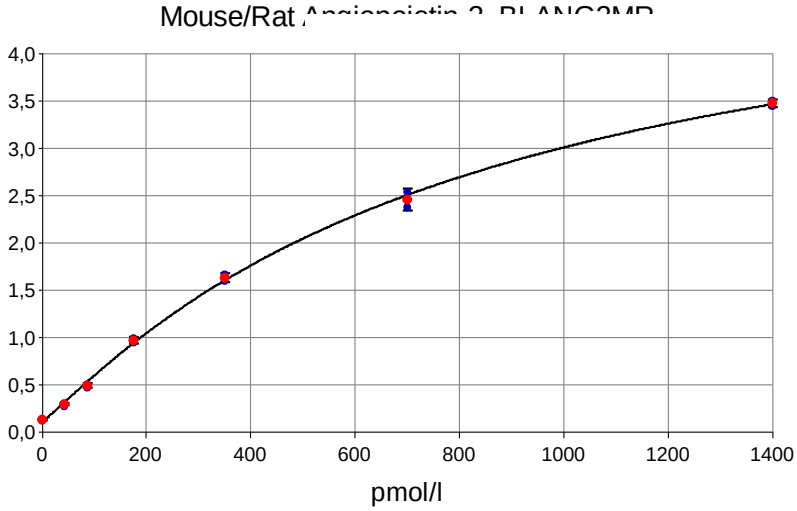
Method	Sandwich ELISA, HRP/TMB, 12x8-well detachable strips			
Sample type	Mouse or rat serum, plasma			
Standard range	0 – 1400 pmol/l (0 – 76,860 pg/ml)			
Conversion factor	1 pg/ml = 0.018 pmol/l (MW: 54.9 kDa)			
Sensitivity	LOD: 18.3 pmol/l, LLOQ: 21.9 pmol/l			
Sample volume	5 µl / well			
Incubation time and temp.	2 h / 2 h / 1 h / 30 min, room temperature			
Specificity	Endogenous and recombinant mouse/rat Angiopoietin-2.			
Precision	Within-run (n=5) ≤ 4%, In-between-run (n=4) ≤ 9%			
Accuracy		**Average % recovery**		
	Mouse (n=4)	101		
	Rat (n=3)	100		
Dilution linearity of endogenous Angiopoietin-2		**Average % of expected dilution**		
		1+1	**1+3**	**1+7**
	Mouse (n=3)	100	102	101
	Rat (n=4)	92	94	104
Median Angiopoietin-2 values in various cohorts		**n**	**Angiopoietin-2 [pmol/l]**	
	Mouse serum	18	105	
	Mouse plasma	7	127	
	Rat serum (clinical cohort)	8	292	
	Rat plasma	11	127	

PRODUCT OVERVIEW

The Angiopoietin-2 Mouse/Rat immunoassay is a 5.5 hour, 96-well sandwich ELISA for the quantitative determination of mouse or rat Angiopoietin-2 in serum or plasma.

TYPICAL STANDARD CURVE

The figure below shows a typical standard curve for the Angiopoietin-2 Mouse/Rat ELISA. The immunoassay is calibrated against recombinant mouse Angiopoietin-2 peptide:

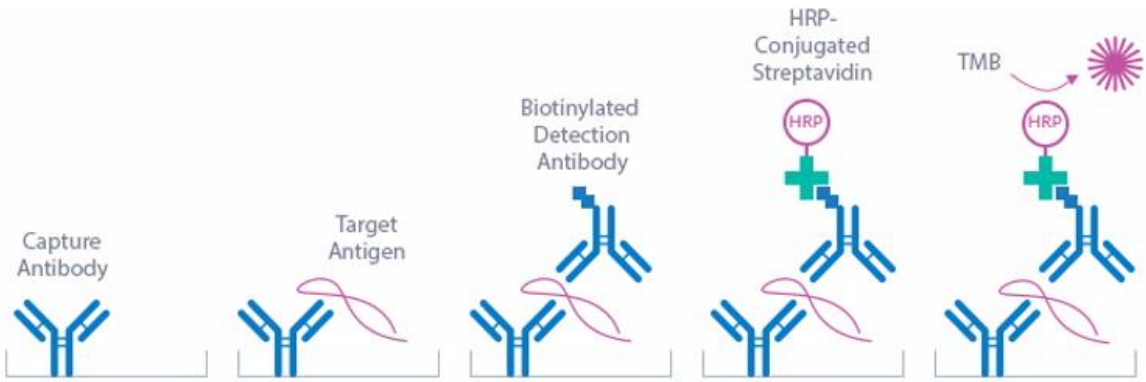


CALIBRATION

The Angiopoietin-2 Mouse/Rat ELISA is calibrated against mouse Angiopoietin-2 protein (https://www.uniprot.org/uniprot/O35608).

PRINCIPLE OF THE ASSAY

The Angiopoietin-2 Mouse/Rat ELISA kit is a sandwich enzyme immunoassay for the quantitative determination of Angiopoietin-2 in mouse or rat samples. The kit utilizes recombinant mouse Angiopoietin-2 as a calibrator. Mouse, rat, bovine, and human Angiopoietin-2 share a high homology (>85%).



Target antigen: mouse/rat Angiopoietin-2
Calibrator: recombinant mouse Angiopoietin-2

This kit is a sandwich enzyme immunoassay for the quantitative determination of mouse/rat Angiopoietin-2 in serum and plasma samples. In a first step, STD/CTRL/Sample are pipetted into the wells of the microtiter strips, which are pre-coated with a recombinant monoclonal Angiopoietin-2 antibody. Angiopoietin-2 present in the sample binds to the pre-coated antibody in the well. After a first washing step, which removes non-specifically unbound material, detection antibody is added and forms a sandwich with the antigen bound on the plate. After another washing step, the conjugate (streptavidin-HRP) is pipetted into the wells and reacts with the detection antibody. After the third and last washing step, the substrate (TMB, tetramethylbenzidine) is pipetted into the wells. The enzyme catalysed color change of the substrate is directly proportional to the amount of mouse/rat Angiopoietin-2 present in the sample. This color change is detectable with a standard microtiter plate ELISA reader. The concentration of Angiopoietin-2 in the sample is determined directly from the dose response curve.

SAMPLE VALUES

Angiopoietin-2 Values

To provide reference values for circulating mouse and rat Angiopoietin-2, a panel of samples was tested. A summary of the results is shown below:

		Angiopoietin-2 [pmol/l]			
Sample matrix	**n**	**Mean**	**Median**	**Minimum**	**Maximum**
Mouse serum	18	132	**105**	57	457
Mouse plasma	7	133	**127**	94	207
Rat serum	8	293	**292**	220	354
Rat plasma	11	136	**127**	107	178

It is recommended to establish the normal range for each laboratory.

ASSAY PERFORMANCE CHARACTERISTICS

ACCURACY

The accuracy of an ELISA is defined as the precision with which it can recover samples of known concentrations.

The recovery of the Angiopoietin-2 Mouse/Rat ELISA was measured by adding recombinant mouse Angiopoietin-2 to mouse and rat samples containing a known concentration of endogenous Angiopoietin-2. The %recovery of the spiked concentration was calculated as the percentage of measured compared over the expected value.

This table shows the summary of the recovery experiments in different sample matrices:

		% Recovery			
Sample Matrix	**n**	**+150 pmol/l**		**+750 pmol/l**	
		Mean	**Range**	**Mean**	**Range**
Mouse	4	101	93-107	94	89-99
Rat	3	100	74-121	94	91-98

Experiments:
Recovery of spiked samples was tested by adding two concentrations of mouse recombinant Angiopoietin-2 (150 pmol/l and 750 pmol/l) to mouse and rat samples.

Data showing recovery of recombinant mouse ANG2 in mouse serum samples:

Sample ID	Spike ANG2 [pmol/l]			S/R [%]	
	0	**150**	**750**	**150**	**750**
MS1	180	326	807	107	96
MS2	176	312	832	101	99
MS3	133	276	752	103	91
MS4	147	275	744	93	89
			Mean S/R [%]	**101**	**94**
			Min	93	89
			Max	107	99

Data showing recovery of recombinant mouse ANG2 in rat plasma samples:

Sample ID	Spike ANG2 [pmol/l]			S/R [%]	
	0	**150**	**750**	**150**	**750**
RP1	185	350	827	121	98
RP2	141	289	770	106	93
RP3	138	238	749	74	91
			Mean S/R [%]	**100**	**94**
			Min	74	91
			Max	121	98

DILUTION LINEARITY & PARALLELISM

Tests of dilution linearity and parallelism ensure that both endogenous and recombinant samples containing Angiopoietin-2 behave in a dose dependent manner and are not affected by matrix effects. Dilution linearity assesses the accuracy of measurements in diluted clinical samples spiked with known concentrations of recombinant analyte. By contrast, parallelism refers to dilution linearity in clinical samples and provides evidence that the endogenous analyte behaves in same way as the recombinant one.

Dilution linearity

Experiments:
Dilution linearity was assessed by serially diluting samples spiked with 650 pmol/l or 750 pmol/l recombinant mouse Angiopoietin-2 with assay buffer.

Summary table below shows the mean recovery and range of serially diluted recombinant mouse Angiopoietin-2 in several sample matrices:

Sample matrix	n	Recovery [%]					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Mouse	4	103	87-116	116	112-119	113	98-122
Rat	3	90	88-92	79	10-84	-	-

Detailed data of recovery of recombinant mouse Angiopoietin-2 in diluted samples are shown below:

Data showing dilution linearity of 650 pmol/l recombinant mouse Angiopoietin-2 (ANG2) spiked into mouse serum samples (ref) containing endogenous ANG2:

Sample ID	ANG2 [pmol/l]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
MS1	594	278	166	73	94	112	98
MS2	596	259	172	91	87	116	122
MS3	533	308	155	80	116	116	120
MS4	571	331	169	82	116	119	115
				Mean R [%]	**103**	**116**	**113**
				Min	87	112	98
				Max	116	119	122

Data showing dilution linearity of 750 pmol/l recombinant mouse Angiopoietin-2 (ANG2) spiked into rat plasma (ref) containing endogenous ANG2:

Sample ID	ANG2 [pmol/l]			Recovery [%]	
	Ref	1+1	1+3	1+1	1+3
RP1	827	381	174	92	84
RP2	770	338	135	88	70
RP3	749	335	157	89	84
			Mean R [%]	**90**	**79**
			Min	88	70
			Max	92	84

Parallelism

Experiment:
Parallelism was assessed by serially diluting samples containing **endogenous** Angiopoietin-2 (ANG2) with assay buffer.

Summary table below show the mean recovery and range of serially diluted endogenous ANG2 in several sample matrices:

Sample matrix	n	Recovery [%]					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Mouse	3	100	96-104	102	97-110	101	87-109
Rat	4	92	83-98	94	89-99	104	98-112

Detailed data of recovery of endogenous Angiopoietin-2 (ANG2) in diluted samples are shown below:

Data showing parallelism of endogenous ANG2 in mouse serum samples:

Sample ID	ANG2 [pmol/l]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
MS1	449	233	123	61	104	110	109
MS2	207	104	52	22	100	100	87
MS3	143	69	35	19	96	97	108
				Mean R [%]	**100**	**102**	**101**
				Min	96	97	87
				Max	104	110	109

Data showing parallelism of endogenous ANG2 in rat plasma samples:

Sample ID	ANG2 [pmol/l]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
RP1	147	72	36	19	98	99	102
RP2	150	68	33	18	90	89	98
RP3	108	53	27	14	98	99	104
RP4	145	60	33	20	83	91	112
				Mean R [%]	**92**	**94**	**104**
				Min	83	89	98
				Max	98	99	112

PRECISION

The precision of an ELISA is defined as its ability to measure the same concentration consistently within the same experiments carried out by one operator (within-run precision or repeatability) and across several experiments using the same samples but conducted by several operators using different ELISA lots (in-between-run precision or reproducibility).

Within-Run Precision (Repeatability or Intra-Assay)

Experiment:
Within-run / intra-assay precision was assessed by measuring 2 samples of known concentrations 5 times within 1 kit lot by 1 operator.

Within-run (n=5)	Sample 1	Sample 2
Mean (pmol/l)	77	120
SD (pmol/l)	3.0	2.2
CV (%)	4	2

In-Between-Run Precision (Reproducibility or Inter-Assay)

Experiment:
In-between-run / inter-assay precision was assessed by measuring 2 samples 5 times within 2 kit lots by 2 different operators.

In-between run (n=4)	Sample 1	Sample 2
Mean (pmol/l)	144	182
SD (pmol/l)	4.5	16.6
CV (%)	3	9

DETECTION LIMIT & SENSITIVITY

To determine the sensitivity of the Angiopoietin-2 mouse/rat ELISA, experiments measuring the Lower Limit of Detection (LOD) and the Lower Limit of Quantification (LLOQ) were conducted.

The LOD, also called the detection limit, is the lowest point at which a signal can be distinguished above the background signal, i.e. the signal that is measured in the absence of mouse Angiopoietin-2, with a confidence level of 99%. It is defined as the mean back calculated concentration of standard 1 (0 pmol/l of mouse Angiopoietin-2, five independent measurements) plus three times the standard deviation of the measurements.

The LLOQ, or sensitivity of an assay, is the lowest concentration at which an analyte can be accurately quantified. The criteria for accurate quantification at the LLOQ are an analyte recovery between 75% and 125% and a coefficient of variation (CV) of less than 25%. To determine the LLOQ, standard 2, i.e. the lowest standard containing mouse Angiopoietin-2, is diluted, measured five times and its concentration is back calculated. The lowest dilution, which meets both criteria, is reported as the LLOQ.

The following values were determined for the Angiopoietin-2 Mouse/Rat ELISA:

LOD	**18.3 pmol/l**
LLOQ	**21.9 pmol/l**

SAMPLE STABILITY

Sample Collection and Storage
Serum and plasma are suitable for use in this assay. Do not change sample type during studies. We recommend duplicate measurements for all samples, standards and controls. The sample collection and storage conditions listed are intended as general guidelines.

Blood samples:
Collect venous blood samples by using standardized blood collection tubes for serum or plasma. Perform serum and plasma separation by centrifugation according to supplier's instructions of the blood collection devices and measure the acquired serum or plasma samples as soon as possible or aliquot and store at -25°C or lower.

Freeze-Thaw Stability of Samples Containing Endogenous Angiopoietin-2
The stability of endogenous Angiopoietin-2 was tested by comparing measurements in blood samples that had undergone 4 freeze-thaw cycles (F/T). The mean recovery of sample concentration after 4 freeze-thaw cycles is 96%. Samples can undergo at least up to 4 freeze-thaw cycles.

STANDARD STABILITY

The stability of recombinant mouse Angiopoietin-2 (ANG2) was tested by comparing 3 measurements of standards spiked to different values that had undergone 5 freeze-thaw cycles.

The mean recovery of standard concentration after 5 freeze-thaw cycles is 99%.

	ANG2 [pmol/l]				% Recovery after 5 F/T cycles
Sample matrix	**Ref**	**1x**	**3x**	**5x**	
Standard #1	1374	1383	1387	1398	102
Standard #2	634	643	578	609	96
				Mean R [%]	**99**

Standards can undergo at least up to 5 freeze-thaw cycles.

The stability of recombinant mouse Angiopoietin-2 (ANG2) was tested by comparing 3 measurements in standards spiked to different values that was tested for bench top stability for 2h, 4h and 20h at room temperature (18-26°C).

The mean recovery of standard concentration after 20 hours at room temperature is 99%.

	ANG2 [pmol/l]				% Recovery after 20h @ RT
Sample matrix	**Ref**	**2h**	**4h**	**20h**	
Standard #1	1394	1427	1437	1434	103
Standard #2	676	670	659	636	94
				Mean R [%]	**99**

SPECIFICITY

The specificity of an ELISA is defined as its ability to exclusively recognize the analyte of interest.

Competition of Signal
Competition experiments were carried out by pre-incubating mouse and rat samples with an excess of coating antibody. The concentration measured in this mixture was then compared

to a reference value, which was obtained from the same sample but without the pre-incubation step. Mean competition was 98%.

ID	ANG2 [pmol/l]		Recovery [%] Competition
	Reference	Reference + CAB	
M1	449	6	99
M2	207	0	100
M3	61	0	100
M4	143	0	100
R1	188	19	90
R2	145	0	100
R3	142	0	100
R4	146	6	96
		Mean Comp. [%]	**98**

Additional Documents Available Online (www.bmgrp.com)

Instructions for Use (IFU, package insert)
Material Safety Data Sheet (MSDS)