

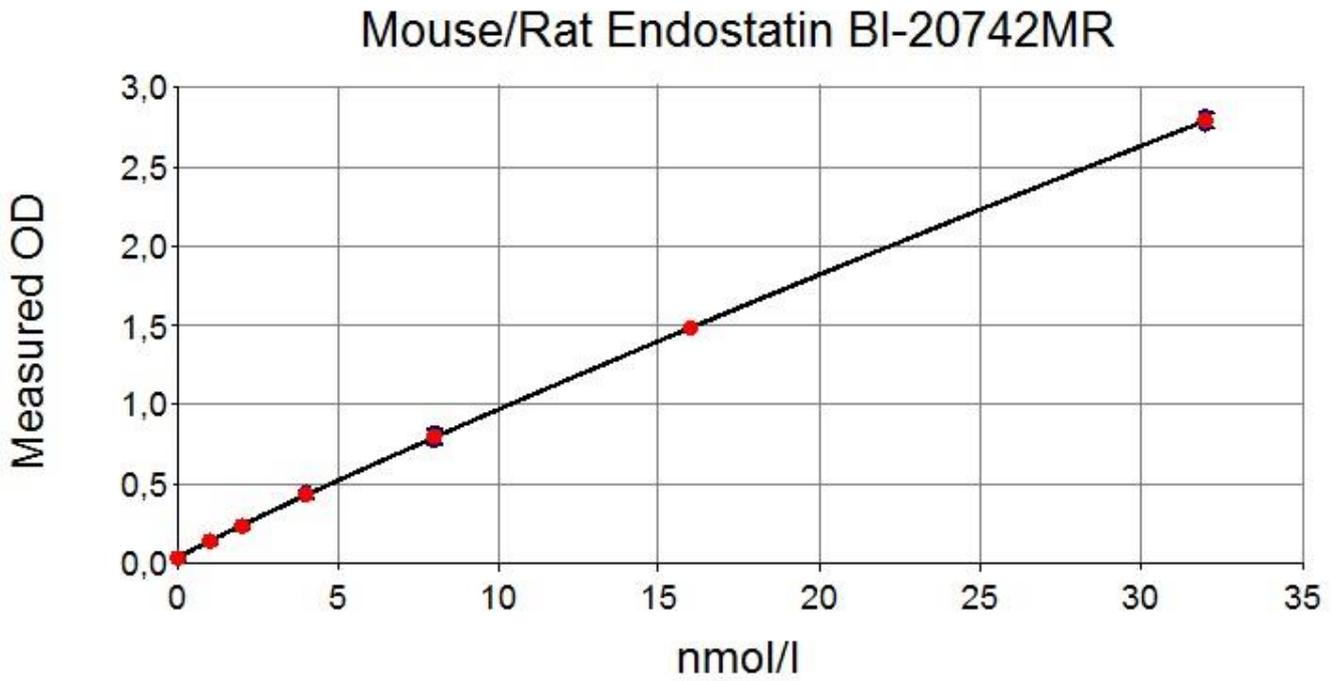
Endostatin Mouse/Rat ELISA (Cat.No. BI-20742MR)
For the Determination of Endostatin in Mouse/Rat Samples

ASSAY CHARACTERISTICS

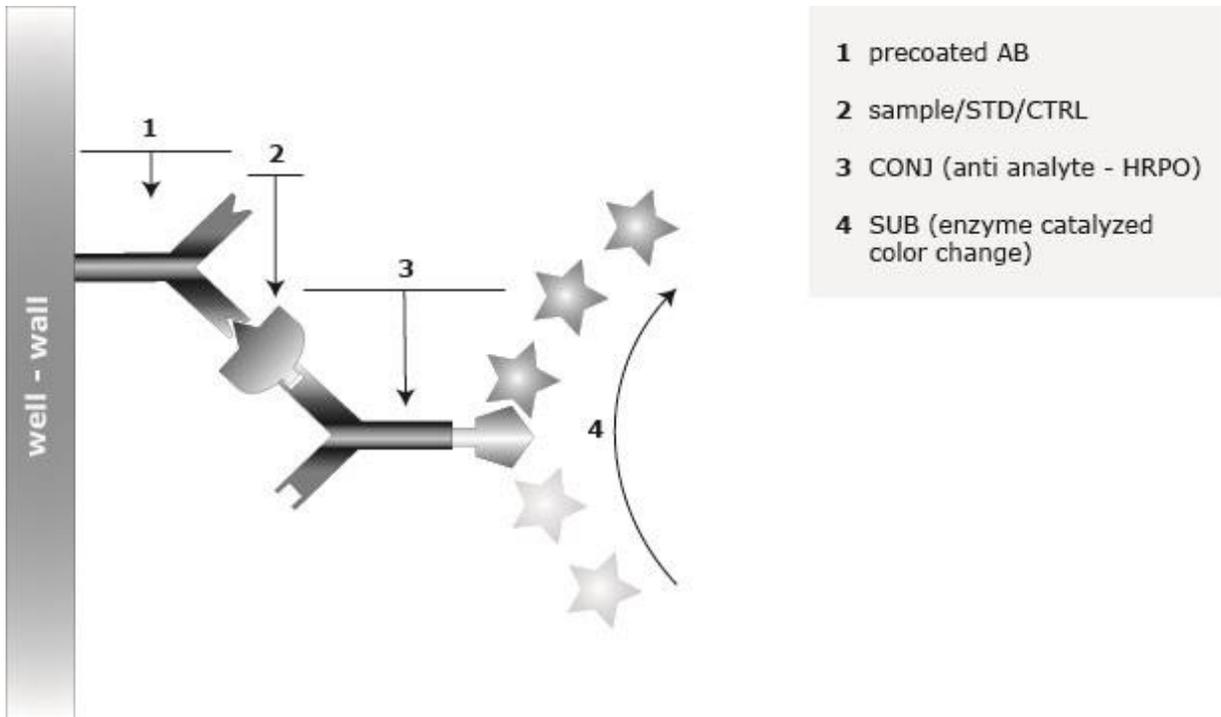
| | | | | |
|--|--|-------------------------------------|-----------|----------|
| Method: | Sandwich ELISA, HRP/TMB, 12x8-well strips | | | |
| Sample type: | Mouse or rat serum, plasma | | | |
| Standard range: | 0-32 nmol/l (0 / 1 / 2 / 4 / 8 / 16 / 32) | | | |
| Conversion factor: | 1 ng/ml = 0.049 nmol/l or 1 nmol/l=20.376 ng/ml (MW: 20.4 kDa) | | | |
| Sample volume: | 5 µl / sample | | | |
| Incubation time: | 2 h / 30 min – room temperature | | | |
| Sensitivity: | LOD (0 nmol/l + 3 SD): 0.24 nmol/l; LLOQ: 0.5 nmol/l | | | |
| Specificity: | This assay detects recombinant and endogenous mouse and rat Endostatin. | | | |
| Precision: | Intra-assay (n=5) ≤ 9%, Inter-assay (n=15) ≤ 10% | | | |
| Spike/Recovery (average recovery spiked with 25 nmol/l recombinant mouse Endostatin): | Mouse serum (n=7): 95% | Rat serum (n=4): 97% | | |
| | Mouse plasma (n=5): 91% | Rat plasma: n.a. | | |
| Dilution linearity of recombinant and endogenous Endostatin (average recovery of expected Endostatin values after a 1+1; 1+3; 1+7 dilution in ASYBUF): | Recovery (%): | Endostatin recombinant / endogenous | | |
| | Dilution: | 1+1 | 1+3 | 1+7 |
| | Mouse serum (n=6) | 108 / 101 | 117 / 88 | 114 / * |
| | Mouse plasma (n=5) | 101 / 107 | 104 / 109 | 103 / * |
| | Rat serum (n=7) | 99 / 96 | n.a. / 89 | n.a. / * |
| | Rat plasma (n=4) | n.a. / 89 | n.a. / 80 | n.a. / * |
| Values from various mouse and rat samples: | Mouse sera C57BL6J0laHsd, 12 weeks (n=11): 6.7 ± 0.8 nmol/l Wildtype normal mouse sera, 12 weeks, male (n=10): 5.4 ± 1.2 nmol/l Wildtype normal rat sera, 12 weeks, male (n=8): 2.5 ± 0.4 nmol/l <i>Each laboratory should establish its own reference range for the samples under investigation.</i> | | | |

*not detectable, n.a.: not analysed; homology of mouse and rat Endostatin is estimated to be 95.7%

Typical standard curve of Biomedica Endostatin mouse/rat ELISA:



Principle of the assay:



Homology of mouse and rat Endostatin

Homology of mouse and rat Endostatin is estimated to be 95.7%. The antibodies utilized in this assay are both polyclonal antibodies that detect various regions of the Endostatin molecule.

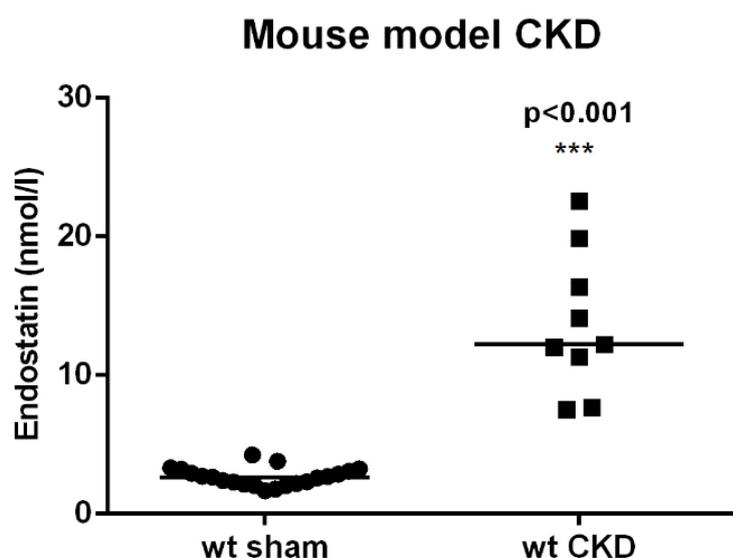
| | | | |
|-------|-----|--|-----|
| Mouse | 1 | HTHQDFQPVHLHLVALNTPLSGGMRGIRGADFQCFQQARAVGLSGTFRAFL | 50 |
| | | . | |
| Rat | 1 | HTHQDFHFPVHLHLVALNTPLSGGMRGIRGADFQCFQQARAVGLSGTFRAFL | 50 |
| Mouse | 51 | SSRLQDLYSIVRRADRGSVPIVNLKDEVLSPSWDSLFSGSQGQLQPGARI | 100 |
| | | | |
| Rat | 51 | SSRLQDLYSIVRRADRSSVPIVNLKDEVLSPSWDTLFSGSQGQLHSGARI | 100 |
| Mouse | 101 | FSFDGRDVLRRHPAWPQKSVWHGSDPSGRRLMESYCETWRTETTGTATGQAS | 150 |
| | | | |
| Rat | 101 | FSFDGRDVLRRHPAWPQKSVWHGSDPSGRRLMESYCETWRTEATGVTGQAS | 150 |
| Mouse | 151 | SLLSGRLLQKAASCHNSYIVLCIENSFMTSFSK | 184 |
| | | | |
| Rat | 151 | SLLSGRLLQKAESCHNSYIVLCIENSFMTSFSK | 184 |

Comparison of mouse to rat Endostatin that share a 95.7 % homology in their amino acid sequence.

Endostatin values from "normal" control mouse/rat serum samples:

| Samples | Age, gender | n | Endostatin [nmol/l] |
|-------------------------------------|----------------|----|---------------------|
| Mouse sera (C57BL6J0laHsd) controls | 12 wks | 11 | 6.7 ± 0.8 |
| Wildtype mouse sera normal values | 12 wks, female | 10 | 4.1 ± 0.6 |
| Wildtype mouse sera normal values | 12 wks, male | 10 | 5.4 ± 1.2 |
| Wildtype rat sera normal values | 12 wks, male | 8 | 2.5 ± 0.4 |

Endostatin serum values: mouse chronic kidney disease (CKD) model:

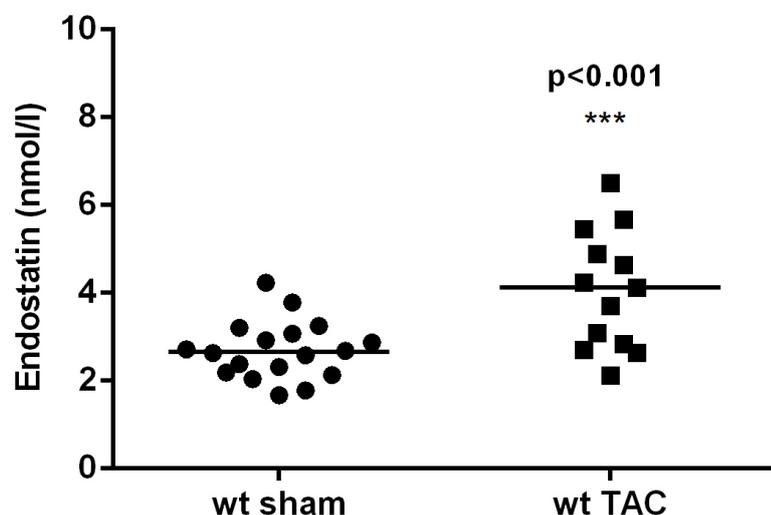


- Mean Endostatin serum values from wildtype normal mice. Age: 6 months, male (n= 21): 2, 594 ± 0,395 nmol/l
- Mean Endostatin serum values from wildtype CKD mice. Age: 6 months,

male (n= 9): 14,005 ± 1,740 (pmol/ml)

Endostatin serum values: mouse thoracic aortic constriction (TAC) model:

Mouse model TAC



PERFORMANCE CHARACTERISTICS

Spike Recovery:

Recovery of mouse Endostatin in mouse and rat samples was evaluated. The spiked samples were diluted 1+50 as indicated in the package insert.

Summary

| Matrix | n | Endostatin (+8 nmol/l) | | Endostatin (+25 nmol/l) | |
|--------------|---|------------------------|---------|-------------------------|---------|
| | | Mean | Range | Mean | Range |
| Mouse serum | 7 | 97% | 86-112% | 95% | 85-111% |
| Mouse plasma | 5 | 85% | 78-89% | 91% | 83-96% |

| Matrix | n | Endostatin (+7.4 nmol/l) | | Endostatin (+22 nmol/l) | |
|-----------|---|--------------------------|--------|-------------------------|---------|
| | | Mean | Range | Mean | Range |
| Rat Serum | 4 | 83% | 78-95% | 97% | 95-100% |

Spike recovery experiments in mouse samples:

Recovery of spiked samples was tested by adding mouse recombinant Endostatin to different mouse serum and plasma samples. The spiked samples were diluted 1+50 as indicated in the package insert.

Data showing spike/recovery of mouse serum samples

| Sample ID | Spike Endostatin [nmol/l] | | | S/R [%] | |
|---------------------|---------------------------|-------|-------|-----------|-----------|
| | 0 | 8 | 25 | 8 | 25 |
| #MS1 | 3.63 | 11.19 | 30.38 | 98 | 111 |
| #MS2 | 3.01 | 10.35 | 25.34 | 95 | 93 |
| #MS3 | 3.30 | 12.01 | 27.35 | 112 | 100 |
| #MS4 | 2.78 | 9.75 | 24.37 | 90 | 89 |
| #MS5 | 3.49 | 10.72 | 26.02 | 94 | 94 |
| #MS6 | 2.49 | 9.18 | 23.13 | 86 | 85 |
| #MS7 | 9.14 | 16.66 | 29.74 | 103 | 92 |
| Mean S/R [%] | | | | 97 | 95 |

Data showing spike/recovery of mouse plasma samples

| Sample ID | Spike Endostatin [nmol/l] | | | S/R [%] | |
|---------------------|---------------------------|------|-------|-----------|-----------|
| | 0 | 8 | 25 | 8 | 25 |
| #MP1 | 1.99 | 8.64 | 24.50 | 85 | 92 |
| #MP2 | 1.64 | 8.24 | 25.12 | 84 | 96 |
| #MP3 | 1.65 | 8.49 | 21.96 | 87 | 83 |
| #MP4 | 1.50 | 8.46 | 24.38 | 89 | 93 |
| #MP5 | 2.37 | 8.41 | 24.05 | 78 | 89 |
| Mean S/R [%] | | | | 85 | 91 |

Spike recovery experiments in rat samples:

Recovery of spiked samples was tested by adding recombinant mouse Endostatin to rat serum samples. The spiked samples were diluted 1+50 as indicated in the package insert.

Data showing spike/recovery of rat serum samples

| Sample ID | Spike Endostatin [nmol/l] | | | S/R [%] | |
|---------------------|---------------------------|------|-------|-----------|-----------|
| | 0 | 7.4 | 22 | 7.4 | 22 |
| #RS1 | 2.90 | 8.8 | 23.80 | 80 | 95 |
| #RS2 | 4.10 | 10.1 | 25.10 | 81 | 95 |
| #RS3 | 3.40 | 10.4 | 25.50 | 95 | 100 |
| #RS4 | 2.10 | 7.9 | 23.60 | 78 | 98 |
| Mean S/R [%] | | | | 83 | 97 |

Dilution Linearity:

Dilution linearity was assessed in mouse and rat samples containing *endogenous* Endostatin and in samples that were spiked with 25 nmol/l and 22 nmol/l of recombinant mouse Endostatin.

Summary

| Dilution Factor | R [%] | Mouse Serum | | Mouse Plasma | |
|-----------------|---------------|-------------------|------------------|-------------------|------------------|
| | | Recombinant (n=7) | Endogenous (n=6) | Recombinant (n=5) | Endogenous (n=6) |
| 1+1 | Mean Range | 108 103-119 | 101 95-112 | 101 96-113 | 107 94-114 |
| 1+3 | Mean Range | 117 107-130 | 88 75-96 | 103 101-105 | 109 85-133 |
| 1+7 | Mean Range | 114 108-124 | * | 103 95-107 | * |

*not detectable

| Dilution Factor | R [%] | Rat Serum | | Rat Plasma | |
|-----------------|---------------|-------------------|------------------|-------------|------------------|
| | | Recombinant (n=7) | Endogenous (n=7) | Recombinant | Endogenous (n=4) |
| 1+1 | Mean Range | 99 78-108 | 96 89-101 | n.a. | 89 80-95 |
| 1+3 | Mean Range | n.a. | 89 78-105 | n.a. | 80 75-89 |
| 1+7 | Mean Range | n.a. | * | n.a. | * |

*not detectable, n.a.: not analysed

Mouse Experiments:

Dilution linearity of the endogenous analyte:

Dilution linearity was assessed by diluting mouse serum and plasma samples 1+1, 1+3 with assay buffer. The sample preparation was performed according to the assay protocol.

Data showing the dilution of *endogenous* mouse Endostatin in mouse serum samples

| Sample ID | Endostatin [nmol/l] | | | R [%] | |
|-----------|---------------------|------|-------------------|------------|-----------|
| | Reference | 1+1 | 1+3 | 1+1 | 1+3 |
| #MS1 | 3.79 | 1.90 | 0.71 | 100 | 75 |
| #MS2 | 4.74 | 2.32 | 1.09 | 98 | 92 |
| #MS3 | 4.22 | 2.19 | 0.92 | 104 | 87 |
| #MS4 | 3.94 | 1.94 | 0.90 | 98 | 91 |
| #MS5 | 2.68 | 1.50 | 0.64 | 112 | 96 |
| #MS6 | 5.01 | 2.39 | 1.13 | 95 | 90 |
| | | | Mean R [%] | 101 | 88 |

Data showing the dilution of *endogenous* mouse Endostatin in mouse plasma samples

| Sample ID | Endostatin [nmol/l] | | | R [%] | |
|-------------------|---------------------|------|------|------------|------------|
| | Reference | 1+1 | 1+3 | 1+1 | 1+3 |
| #MP1 | 5.52 | 2.59 | 1.42 | 94 | 103 |
| #MP2 | 5.43 | 2.79 | 1.47 | 103 | 108 |
| #MP3 | 1.99 | 1.08 | 0.66 | 109 | 133 |
| #MP4 | 1.65 | 0.92 | 0.43 | 112 | 104 |
| #MP5 | 1.50 | 0.84 | 0.32 | 112 | 85 |
| #MP6 | 2.37 | 1.35 | 0.73 | 114 | 123 |
| Mean R [%] | | | | 107 | 109 |

Dilution linearity of the recombinant analyte:

Dilution linearity was assessed by spiking mouse serum and plasma samples with 25 nmol/l recombinant mouse Endostatin. The spiked samples were diluted 1+1, 1+3, 1+7 with assay buffer. The sample preparation was performed according to the assay protocol.

Data showing the dilution of *recombinant* mouse Endostatin in mouse serum samples

| Sample ID | Endostatin [nmol/l] | | | | R [%] | | |
|-------------------|---------------------|-------|------|------|------------|------------|------------|
| | Spike +25 nmol/l | 1+1 | 1+3 | 1+7 | 1+1 | 1+3 | 1+7 |
| #MS1 | 30.09 | 17.84 | 9.77 | 4.27 | 119 | 130 | 114 |
| #MS2 | 33.82 | 17.81 | 9.06 | 4.57 | 105 | 107 | 108 |
| #MS3 | 30.56 | 16.51 | 8.65 | 4.41 | 108 | 113 | 115 |
| #MS4 | 29.63 | 15.55 | 8.78 | 3.99 | 105 | 119 | 108 |
| #MS5 | 28.98 | 15.41 | 8.41 | 4.49 | 106 | 116 | 124 |
| #MS6 | 28.85 | 15.95 | 8.55 | 4.22 | 111 | 119 | 117 |
| #MS7 | 30.11 | 15.49 | 8.65 | 4.13 | 103 | 115 | 110 |
| Mean R [%] | | | | | 108 | 117 | 114 |

Data showing the dilution of *recombinant* mouse Endostatin in mouse plasma samples

| Sample ID | Endostatin [nmol/l] | | | | R [%] | | |
|-------------------|---------------------|-------|------|------|------------|------------|------------|
| | Spike +25 nmol/l | 1+1 | 1+3 | 1+7 | 1+1 | 1+3 | 1+7 |
| #MP1 | 24.50 | 12.17 | 6.38 | 3.28 | 99 | 105 | 103 |
| #MP2 | 25.12 | 12.06 | 6.12 | 3.14 | 96 | 101 | 103 |
| #MP3 | 21.96 | 12.39 | 6.39 | 3.42 | 113 | 103 | 107 |
| #MP4 | 24.38 | 11.94 | 6.15 | 2.93 | 98 | 103 | 95 |
| #MP5 | 24.05 | 12.24 | 6.19 | 3.25 | 102 | 101 | 105 |
| Mean R [%] | | | | | 101 | 103 | 103 |

Rat Experiments:

Dilution linearity of the endogenous analyte:

Dilution linearity was assessed by diluting rat samples 1+1, 1+3 with assay buffer. The sample preparation was performed according to the assay protocol.

Data showing the dilution of *endogenous* rat Endostatin in rat serum samples

| Sample ID | Endostatin [nmol/l] | | | R [%] | |
|-------------------|---------------------|-----|-----|-----------|-----------|
| | Reference | 1+1 | 1+3 | 1+1 | 1+3 |
| #RS1 | 2.37 | 1.2 | 0.6 | 101 | 94 |
| #RS2 | 2.39 | 1.1 | 0.6 | 92 | 99 |
| #RS3 | 1.81 | 0.8 | 0.4 | 89 | 78 |
| #RS4 | 2.34 | 1.1 | 0.5 | 97 | 83 |
| #RS5 | 2.35 | 1.2 | 0.6 | 100 | 105 |
| #RS6 | 2.23 | 1.1 | 0.6 | 94 | 100 |
| #RS7 | 2.32 | 1.2 | 0.5 | 102 | 91 |
| Mean R [%] | | | | 96 | 89 |

Data showing the dilution of *endogenous* rat Endostatin in rat plasma samples

| Sample ID | Endostatin [nmol/l] | | | | R [%] | | |
|-------------------|---------------------|-----|-----|-----|-----------|-----------|-----------|
| | Reference | 1+1 | 1+2 | 1+4 | 1+1 | 1+2 | 1+4 |
| #RP1 | 3.4 | 1.6 | 1.0 | 0.5 | 93 | 89 | 75 |
| #RP2 | 2.9 | 1.4 | 0.8 | 0.5 | 95 | 78 | 90 |
| #RP3 | 3.4 | 1.4 | 0.9 | 0.4 | 80 | 75 | 54 |
| #RP4 | 3.3 | 1.5 | 0.8 | 0.4 | 89 | 77 | 62 |
| Mean R [%] | | | | | 89 | 80 | 71 |

Dilution linearity of the recombinant analyte:

Dilution linearity was assessed by spiking rat serum with 22 nmol/l recombinant mouse Endostatin. The spiked samples were diluted 1+1 with assay buffer. The sample preparation was performed according to the assay protocol.

Data showing the dilution of *recombinant* mouse Endostatin in rat serum samples

| Sample ID | Endostatin [nmol/l] | | R [%] |
|-------------------|-----------------------------|-------|-----------|
| | Reference [spike 22 nmol/l] | 1+1 | 1+1 |
| #RS1 | 23.80 | 11.10 | 93 |
| #RS2 | 25.10 | 11.60 | 92 |
| #RS3 | 25.50 | 12.70 | 100 |
| #RS4 | 23.60 | 11.00 | 93 |
| #RS5 | 15.30 | 8.30 | 108 |
| #RS6 | 16.30 | 8.60 | 106 |
| #RS7 | 19.00 | 7.40 | 78 |
| Mean R [%] | | | 99 |

Intra-assay precision & Inter-assay precision:

Intra-assay (n=5) ≤ 9%, Inter-assay (n=15) ≤ 10%

Experiment:

Intra-assay: 2 samples of known concentrations were tested 5 times in 1 assay by 1 operator.

Inter-assay: 2 samples of known concentrations were tested 15 times in 3 assays in 3 days by 2 different operators.

Data showing intra-assay and inter-assay precision:

| Intra-assay (n=5) | Sample 1 | Sample 2 | Inter-assay (n=15) | Sample 1 | Sample 2 |
|--------------------------|-----------------|-----------------|---------------------------|-----------------|-----------------|
| Mean (nmol/l) | 0.98 | 31.98 | Mean (nmol/l) | 1.01 | 31.99 |
| SD (nmol/l) | 0.09 | 0.55 | SD (nmol/l) | 0.10 | 0.48 |
| CV (%) | 9 | 2 | CV (%) | 10 | 2 |

The limit of quantification (LOQ):

The LOQ is defined as the mean value of the back calculated concentration plus 3 times the standard deviation. The LOQ of the Endostatin mouse/rat ELISA is 0.24 nmol/l.

The lower limit of quantification (LLOQ):

The lower limit of quantification is defined as the accuracy of the back calculated concentrations and shall not exceed ±25% (acc. to ICH (Ref. 1)).

For the Endostatin mouse/rat ELISA the LLOQ was determined as 0.5 nmol/l.

Specificity:

This assay recognizes endogenous (natural) and recombinant mouse/rat Endostatin.

Experiments:

Samples containing endogenous and recombinant Endostatin were analysed.

The competition was performed by adding a 10fold concentration of the catching antibody.

Competition of a mouse serum sample containing 32 pmol/l recombinant mouse Endostatin

| Sample ID | theor. c [nmol/l] | Reference | | | Competition | | | R comp. [%] |
|------------------|------------------------------|------------------|------------|----------------------|--------------------|------------|----------------------|------------------------|
| | | OD1 | OD2 | Mean [OD] | OD1 | OD2 | Mean [OD] | |
| #MS1 | 32.0 | 2.570 | 2.867 | 2.719 | 0.045 | 0.064 | 0.055 | 98 |

Competition of mouse serum samples containing endogenous mouse Endostatin

| Sample ID | Endostatin [nmol/l] | | R comp. [%] |
|-----------|---------------------|-------------------|-------------|
| | Reference | Competition | |
| # MS1 | 4.02 | 0.00 | 100 |
| # MS2 | 3.79 | 0.00 | 100 |
| # MS3 | 4.74 | 0.00 | 100 |
| # MS4 | 4.22 | 0.00 | 100 |
| # MS5 | 3.41 | 0.00 | 100 |
| # MS6 | 3.94 | 0.00 | 100 |
| # MS7 | 2.68 | 0.00 | 100 |
| # MS8 | 5.01 | 0.00 | 100 |
| | | Mean R [%] | 100 |

Competition of mouse plasma samples containing endogenous mouse Endostatin

| Sample ID | Endostatin [nmol/l] | | R comp. [%] |
|-----------|---------------------|-------------------|-------------|
| | Reference | Competition | |
| # MP1 | 3.1 | 0.0 | 100 |
| # MP2 | 5.5 | 0.3 | 95 |
| # MP3 | 5.4 | 0.3 | 95 |
| # MP4 | 2.0 | 0.1 | 97 |
| # MP5 | 1.6 | 0.0 | 100 |
| # MP6 | 1.7 | 0.0 | 99 |
| # MP7 | 1.5 | 0.0 | 100 |
| # MP8 | 2.4 | 0.1 | 96 |
| | | Mean R [%] | 98 |

Competition of rat serum samples containing endogenous rat Endostatin

| Sample ID | Endostatin [nmol/l] | | R comp. [%] |
|-----------|---------------------|-------------------|-------------|
| | Reference | Competition | |
| # RS1 | 2.4 | 0.00 | 100 |
| # RS2 | 1.8 | 0.00 | 100 |
| # RS3 | 2.3 | 0.00 | 100 |
| # RS4 | 2.3 | 0.00 | 100 |
| # RS5 | 2.2 | 0.00 | 100 |
| | | Mean R [%] | 100 |

Calibration:

This immunoassay is calibrated against purified recombinant mouse Endostatin peptide.

SAMPLE CHARACTERISTICS

Sample stability:

We recommend separating plasma or serum by centrifugation as soon as possible, e.g. 20 min at 2,000 x g, preferably at 4°C (2-8°C). Samples can be stored at 4°C (2-8°C) overnight. For long term storage, aliquot the acquired plasma or serum samples and store at -25°C or lower.

Samples can be subjected to 4 freeze-thaw cycles.

| Sample ID | Endostatin [nmol/l] | | | R [%] |
|-------------------|---------------------|----------|----------|-----------|
| | F/T x+1x | F/T x+3x | F/T x+4x | |
| #S1 | 4.0 | 4.2 | 4.0 | 99 |
| #S2 | 3.6 | 3.7 | 3.0 | 82 |
| #S3 | 4.5 | 4.5 | 3.9 | 87 |
| #S4 | 4.1 | 4.1 | 3.7 | 92 |
| #S5 | 3.2 | 3.5 | 3.0 | 92 |
| #S7 | 3.3 | 3.3 | 3.2 | 99 |
| #S8 | 4.6 | 5.3 | 4.8 | 104 |
| Mean R [%] | | | | 94 |

Validation

The assay is fully validated according to ICH Q2 (R1), Ref. (1).

References

(1) 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”

Available on our homepage

Enzyme immunoassay for the quantitative determination of Endostatin in mouse and rat serum and plasma: Package Insert, MSDS, Information Folder, and References

Version: April 2017