Leucine-rich alpha-2-glycoprotein (LRG) ELISA

for the quantitative determination of leucine-rich alpha-2-glycoprotein in serum, EDTA plasma, heparin plasma, and citrate plasma &
performance check for quantitative determination of LRG in urine samples

Cat. No. BI-LRG. 12 x 8 tests

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

CONTENTS

BIOMEDICA IMMUNOASSAYS

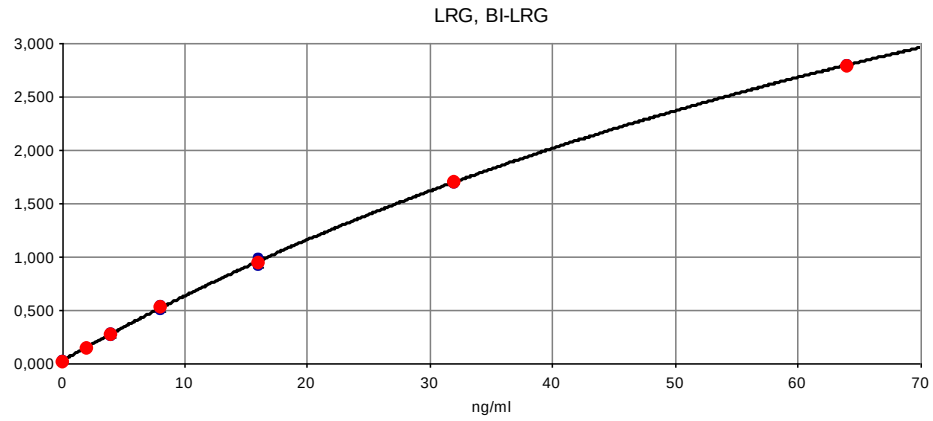
ASSAY CHARACTERISTICS Summary

Method	Sandwich ELISA, HRP/TMB, 12x8-well detachable strips			
Sample type(s)	Serum, EDTA plasma, citrate plasma, heparin plasma (urine and cell culture protocol available)			
Standard range	0 – 64 ng/ml (0 / 2 / 4 / 8 / 16 / 32 / 64)			
Sensitivity	LOD: 0.26 ng/ml; LLOQ: 0.5 ng/ml			
Conversion factor	1 ng/ml = 0.0262 nmol/l; MW: 38.178 kDa			
Sample volume	100 µl pre-diluted sample / well (5 µl sample)			
Assay time	2 h / 1 h / 30 min			
Precision		**n**	**CV (%)**	
	Within-run	3	≤3	
	In-between-run	9	≤6	
Accuracy (Spike/Recovery of recombinant LRG)		**n**	**Recovery (%)**	
			+6.4 ng/ml	**+32 ng/ml**
	Serum	5	86	96
	EDTA plasma	5	85	89
	Heparin plasma	5	90	91
	Citrate plasma	2	96	100
Parallelism of endogenous human LRG		**n**	**Recovery of expected dilution (%)**	
			1+1	**1+3**	**1+7**
	Serum	5	116	117	116
	EDTA plasma	7	107	101	96
	Heparin plasma	1	115	113	109
	Citrate plasma	1	118	111	110
Specificity	Endogenous and recombinant human LRG (leucine-rich alpha-2-glycoprotein)			
Use	Research use only			
Values of apparently healthy donors (performance check for urine samples - page 18)		**n**	**Median LRG (µg/ml)***	
	Serum	18	27.5	
	EDTA plasma	22	27.9	
	Heparin plasma	20	23.8	
	Citrate plasma	22	31.1	

**dilution factor of 1:4000 considered, expressed in µg/ml for better readability*

For further information on assay performance characteristics, matrix comparisons and stability data please find data in this validation data file or contact our customer service by e-mail info@bmgrp.com or by phone +43/1/29107-45.

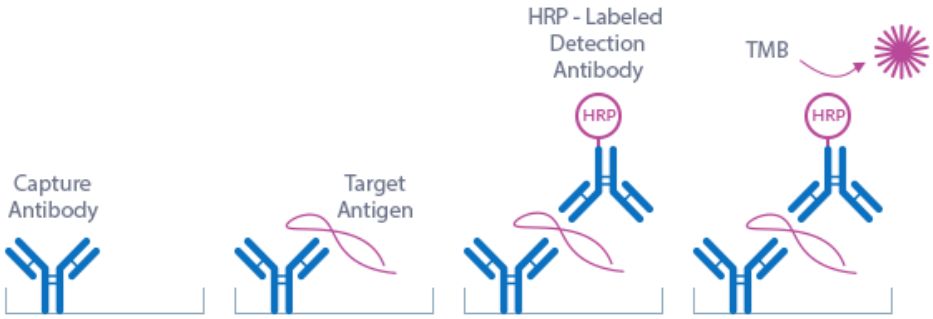
TYPICAL STANDARD CURVE



PRINCIPLE OF THE ASSAY

The leucine-rich alpha-2-glycoprotein (LRG) ELISA kit is a sandwich enzyme immunoassay for the quantitative determination of LRG in serum and plasma (EDTA, heparin, citrate).
A protocol for urine and cell culture supernatants is available.

The figure below explains the principle of the LRG sandwich ELISA:



Capture antibody: polyclonal sheep anti-human LRG antibody
Detection antibody: polyclonal sheep anti-human LRG antibody, HRP-labeled
Target antigen: human LRG (leucine-rich alpha-2-glycoprotein)

In a first step, standards, controls and pre-diluted samples are pipetted into the wells of the microtiter strips, which are pre-coated with polyclonal sheep anti-human LRG antibody. LRG present in the standard/control/sample binds to the pre-coated antibody in the well. All non-specific unbound material is removed in a washing step and the detection antibody (CONJ, polyclonal sheep anti-LRG-HRPO) is pipetted into the wells. After another washing step, the substrate (TMB, tetramethylbenzidine) is added. The enzyme-catalyzed color change of the substrate is directly proportional to the amount of LRG present in the sample. This color change is detectable with a standard microplate reader. A dose response curve of the absorbance (optical density, OD at 450 nm) using the values obtained from the standards versus the standard concentration is generated.
The concentration of LRG in the sample is determined from the dose response curve**.** This sample concentration must be multiplied by the dilution factor used for sample preparation to obtain the final sample concentration.

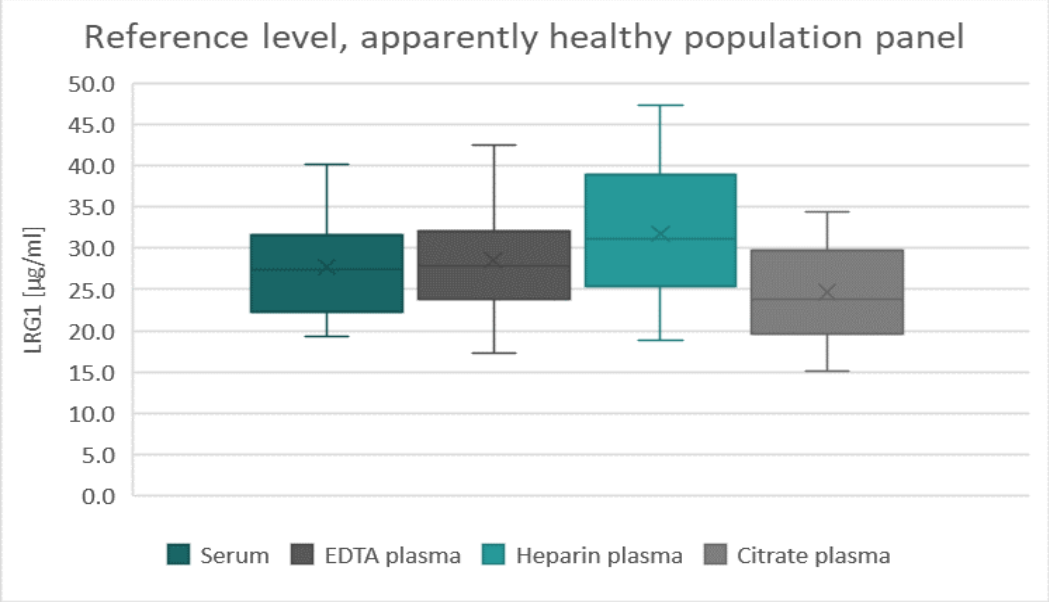
SAMPLE VALUES

Leucine-rich alpha-2-glycoprotein (LRG) in Apparently Healthy Individuals

To provide values for circulating leucine-rich alpha-2-glycoprotein (LRG), a panel of samples from apparently healthy donors was tested. Each individual donated blood for all tested sample matrices.

		LRG [µg/ml]		
Sample Matrix	**n**	**Mean**	**Range**	**Median**
Serum	18	27.7	19.2 - 40.2	27.5
EDTA plasma	22	28.6	17.3 - 42.5	27.9
Heparin plasma	22	24.7	15.1 - 34.4	23.9
Citrate plasma	22	31.8	18.8 - 47.3	31.1

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



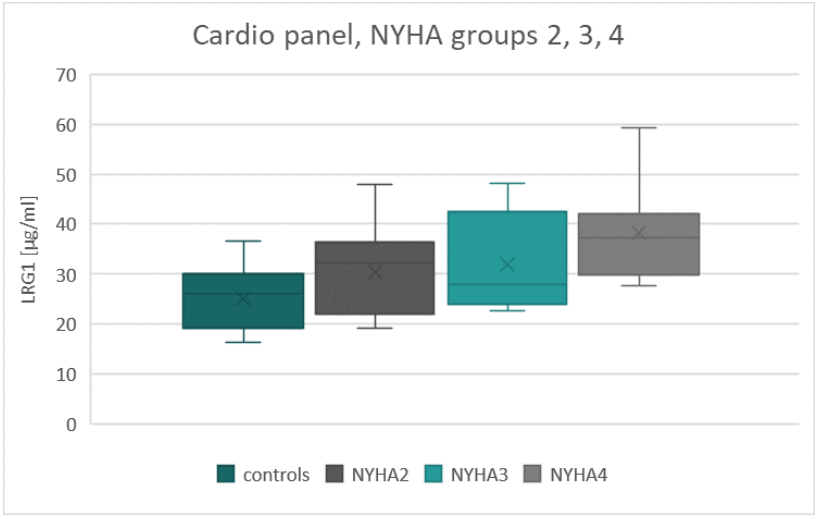
Leucine-rich alpha-2-glycoprotein (LRG) Values in Disease Panels

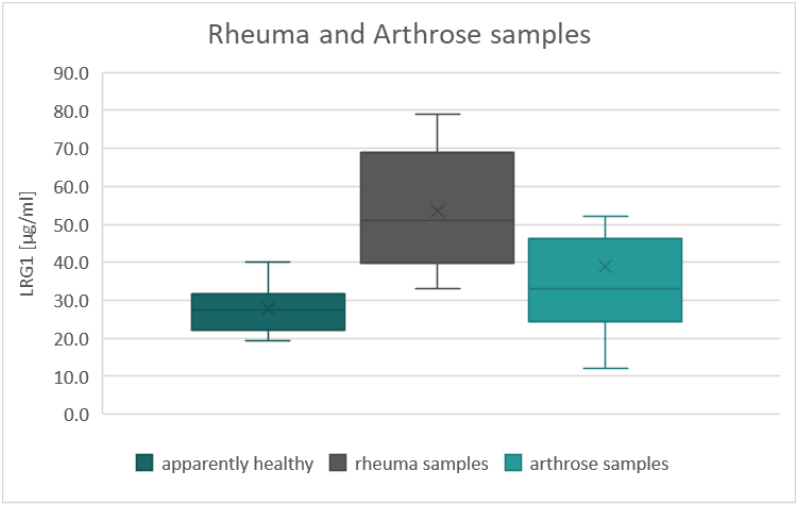
In addition to samples from apparently healthy donors, panels of samples from patients with heart disease (HD), rheuma and arthrose, as well as patients with kidney diseases were tested.

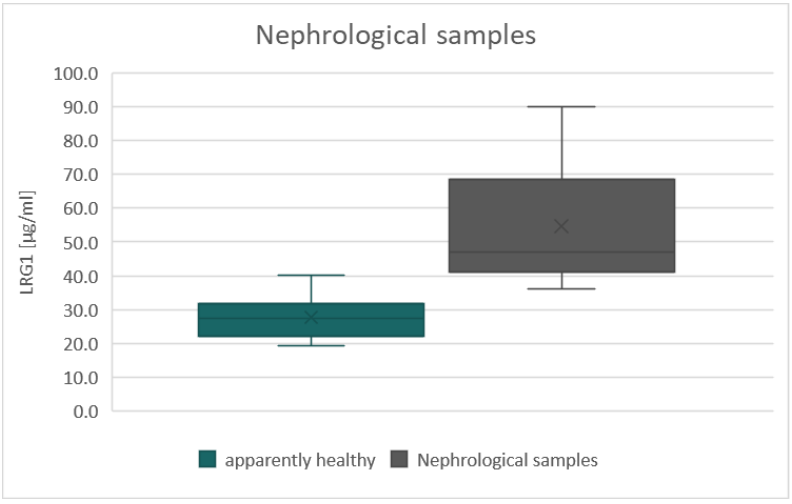
Summary of the results obtained with several disease panels:

		LRG [µg/ml]		
Samples	**n**	**Mean**	**Range**	**Median**
Controls	14	25.0	16.3 - 36.7	26.1
HD - NHYA 2	12	30.4	19.2 - 47.9	32.3
HD -NYHA 3	14	32.0	22.6 - 48.1	27.8
HD -NYHA 4	8	38.3	27.6 - 59.3	37.3
Apparently healthy controls	18	27.7	19.2 - 40.2	27.5

Rheuma cohort	18	53.5	33.0 - 79.0	51.0
Arthrose cohort	16	39	12.0 - 89.0	33.0
Kidney disease	16	54.7	36.0 - 90.0	47.0





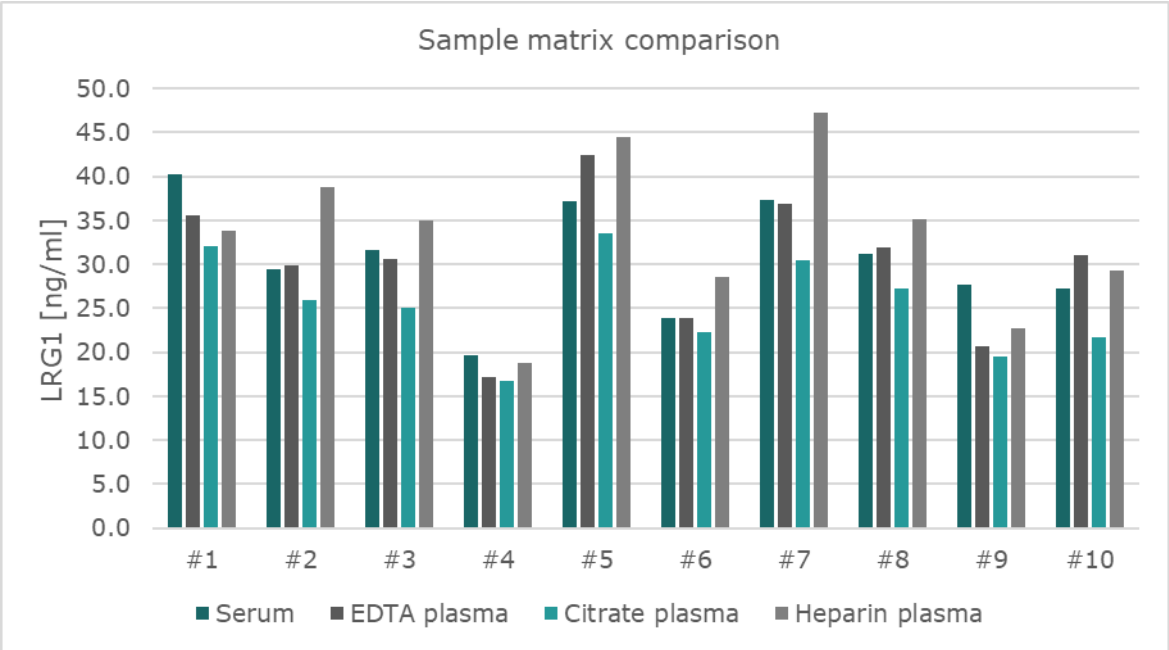


MATRIX COMPARISON

To assess whether all tested matrices behave the same way in the concentrations of leucine-rich alpha-2-glycoprotein (LRG) were measured in serum, EDTA, heparın, and citrate plasma samples prepared from 10 apparently healthy donors. Each individual donated blood in all tested sample matrices.

Data and graph for apparently healthy donors are shown below:

	LRG [µg/ml]				CV [%]
Donor ID	**Serum**	**EDTA plasma**	**Citrate plasma**	**Heparin plasma**	**all matrices**
#1	40.2	35.6	32.1	33.9	8
#2	29.5	29.9	26.0	38.8	15
#3	31.6	30.5	25.1	35.0	12
#4	19.7	17.3	16.7	18.8	7
#5	37.2	42.5	33.5	44.5	11
#6	23.9	23.9	22.2	28.6	10
#7	37.4	36.8	30.4	47.3	16
#8	31.2	32.0	27.3	35.1	9
#9	27.7	20.6	19.5	22.8	14
#10	27.3	31.0	21.6	29.2	13
				Mean CV [%]	**11**



ASSAY PERFORMANCE CHARACTERISTICS

ACCURACY

The precision of an ELISA is defined as its ability to measure the same concentration The accuracy of an ELISA is defined as the precision with which it can recover samples of known concentrations.

The recovery in human samples was tested in the LRG ELISA by adding recombinant LRG to human samples containing a known concentration of endogenous LRG. The %recovery of the spiked concentration was calculated as the percentage of measured over the expected value.

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

Sample Matrix	n	Spike/Recovery [%]			
		+32 ng/ml		+6.4 ng/ml	
		Mean	Range	Mean	Range
Serum	5	96	91 - 99	86	77 - 92
EDTA plasma	5	89	82 - 95	80	65 - 89
Citrate plasma	2	100	99 - 100	96	87 - 106
Heparin plasma	2	91	88 - 94	90	83 - 97

Experiments:
Recovery of spiked samples was tested by adding 2 concentrations of human recombinant LRG to different human sample matrices.

Data showing % recovery of recombinant LRG in human serum samples:

ID	LRG [ng/ml]			S/R [%]	
	Reference	+32 ng/ml	+6.4 ng/ml	+32 ng/ml	+6.4 ng/ml
S1	6.3	38.1	11.3	99	77
S2	6.3	36.7	11.6	95	83
S3	6.4	37.3	12.0	97	88
S4	5.2	36.0	10.8	96	87
S5	4.5	33.7	10.4	91	92
			Mean S/R [%]	**96**	**86**

Data showing % recovery of recombinant LRG in human EDTA plasma samples:

ID	LRG [ng/ml]			S/R [%]	
	Reference	+32 ng/ml	+6.4 ng/ml	+32 ng/ml	+6.4 ng/ml
E1	5.0	35.5	10.7	95	88
E2	7.1	33.4	12.1	82	79
E3	5.9	35.2	11.1	91	81
E4	7.1	33.8	11.3	83	65
E5	3.3	33.0	9.0	93	89
			Mean S/R [%]	**89**	**80**

Data showing % recovery of recombinant LRG in human citrate plasma samples:

ID	LRG [ng/ml]			S/R [%]	
	Reference	+32 ng/ml	+6.4 ng/ml	+32 ng/ml	+6.4 ng/ml
C1	2.5	34.3	9.3	99	106
C2	3.7	35.7	9.3	100	87
			Mean S/R [%]	**100**	**96**

Data showing % recovery of recombinant LRG in human heparin plasma samples:

ID	LRG [ng/ml]			S/R [%]	
	Reference	+32 ng/ml	+6.4 ng/ml	+32 ng/ml	+6.4 ng/ml
H1	3.5	33.7	9.7	94	97
H2	6.0	34.0	11.3	88	83
			Mean S/R [%]	**91**	**90**

DILUTION LINEARITY & PARALLELISM

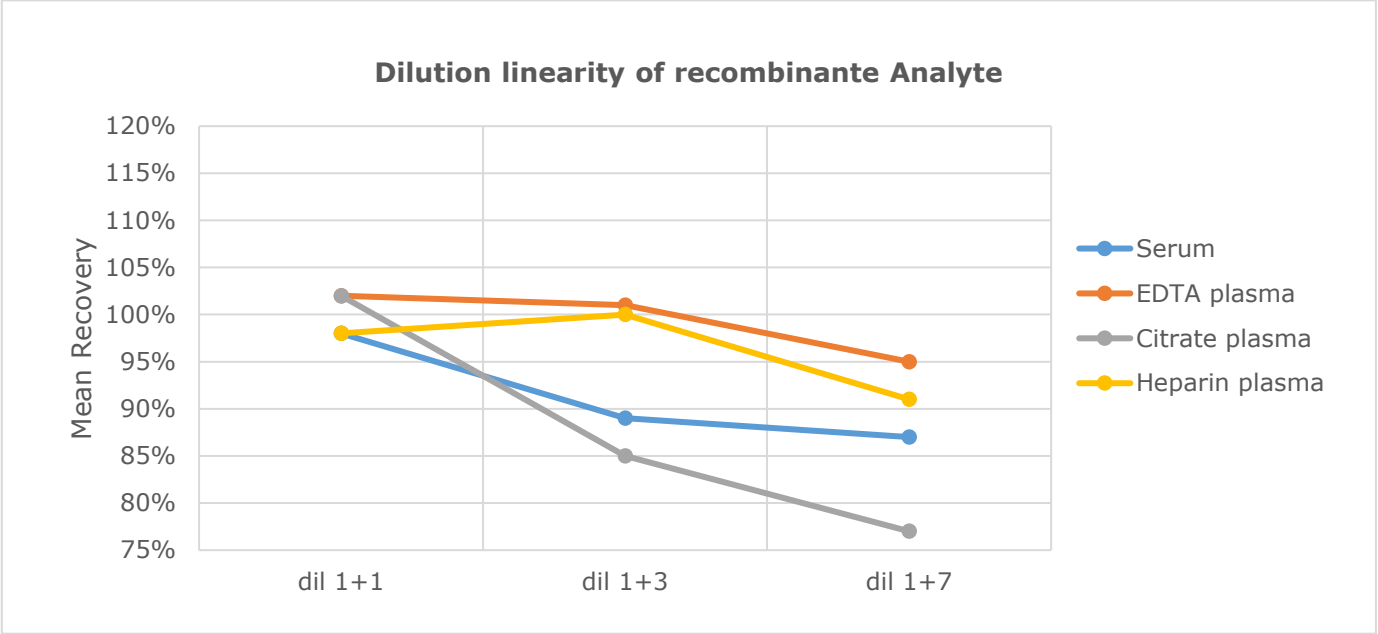
Tests of dilution linearity and parallelism ensure that both, endogenous and recombinant samples containing LRG, behave in a dose-dependent manner and are not affected by matrix effects. Dilution linearity assesses the accuracy of measurements in diluted clinical samples spiked with known concentrations of recombinant analyte. By contrast, parallelism refers to dilution linearity in clinical samples and provides evidence that endogenous analyte behaves in the same way as the recombinant one. Dilution linearity and parallelism are assessed for each sample type and should be within 20% of the expected concentration.

Dilution Linearity

Experiment:
Dilution linearity was assessed by serially diluting samples containing **recombinant** LRG with assay buffer.

The figure and table below show the mean recovery and range of serially diluted recombinant LRG in several sample matrices:

Sample Matrix	n	Recovery [%] of recombinant LRG in diluted samples					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Serum	**6**	98	94 - 103	89	83 - 95	87	75 - 93
EDTA plasma	**6**	102	96 - 110	101	91 - 106	95	91 - 102
Citrate plasma	**2**	102	99 - 104	85	84 - 87	77	76 - 77
Heparin plasma	**2**	98	97 - 99	100	98 - 101	91	89 - 92



Data showing dilution linearity of 32 ng/ml recombinant LRG spiked into human serum and plasma samples (ref):

Calculation of dilution linearity of spiked serum samples:

	LRG [ng/ml]					Recovery [%]		
ID	**Ref +32 ng/ml**	**ref**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
S1	38.1	6.3	18.3	7.9	3.6	96	83	75
S2	36.7	6.3	18.0	7.9	3.9	98	86	85
S3	37.3	6.4	17.5	8.4	4.1	94	90	87
S4	36.0	5.2	16.9	7.8	4.0	94	87	89
S5	33.9	5.8	17.4	8.0	3.9	103	94	93
S6	33.7	4.5	17.2	8.0	3.8	102	95	91
					Mean R [%]	**98**	**89**	**87**

Calculation of dilution linearity of spiked EDTA plasma samples:

	LRG [ng/ml]					Recovery [%]		
ID	**Ref +32 ng/ml**	**ref**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
E1	35.5	5.0	17.1	8.1	4.1	96	91	92
E2	33.4	7.1	18.1	8.9	3.9	109	106	94
E3	35.2	5.9	17.5	8.5	4.2	99	97	95
E4	33.8	7.1	16.8	8.9	4.3	100	106	102
E5	33.0	3.3	16.0	8.2	3.8	97	99	91
E6	31.6	4.9	17.3	8.4	3.7	110	106	95
					Mean R [%]	**102**	**101**	**95**

Calculation of dilution linearity of spiked citrate plasma samples:

	LRG [ng/ml]					Recovery [%]		
ID	**Ref +32 ng/ml**	**ref**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
C1	34.3	2.5	17.9	7.4	3.3	104	87	77
C2	35.7	3.7	17.7	7.5	3.4	99	84	76
					Mean R [%]	**102**	**85**	**77**

Calculation of dilution linearity of spiked heparin plasma samples:

	LRG1 c[ng/ml]					Recovery [%]		
ID	**Ref +32 ng/ml**	**ref**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
H1	33.7	3.5	16.6	8.5	3.9	99	101	92
H2	34.0	6.0	16.5	8.4	3.8	97	98	89
					Mean R [%]	**98**	**100**	**91**

Parallelism

Experiment:
Parallelism was assessed by serially diluting human samples containing **endogenous** LRG with assay buffer.

The table below shows the summary of the mean recovery and range of serially diluted endogenous LRG in several sample matrices:

Sample Matrix	**n**	**% Recovery of endogenous LRG in diluted samples**					
		1+1		**1+3**		**1+7**	
		Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**
Serum	5	116	108 - 123	117	100 - 132	116	110 - 128
EDTA plasma	7	107	101 - 114	101	93 - 111	96	87 - 104
Citrate plasma	1	118		111		110	
Heparin plasma	1	115		113		109	

Data showing dilution linearity of endogenous LRG in human serum samples:

	LRG [ng/ml]				Recovery [%]		
ID	**Reference**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
S1	23.4	13.7	7.0	3.4	117	121	117
S2	25.3	15.5	8.4	4.1	123	132	128
S3	16.0	8.6	4.0	2.2	108	100	111
S4	19.0	10.5	5.6	2.6	110	117	110
S5	20.0	12.1	5.7	2.8	121	114	114
				Mean R [%]	**116**	**117**	**116**

Data showing dilution linearity of endogenous LRG in human EDTA plasma samples:

	LRG [ng/ml]				Recovery [%]		
ID	**Reference**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
E1	19.6	10.1	4.7	2.2	103	97	90
E2	20.2	10.9	5.2	2.5	108	103	99
E3	17.7	9.8	4.4	2.2	110	100	98
E4	18.4	9.8	4.6	2.2	107	100	94
E5	24.0	13.7	6.7	3.1	114	111	104
E6	6.9	3.5	1.7	0.8	101	99	96
E7	14.5	7.3	3.4	1.6	101	93	87
				Mean R [%]	**107**	**101**	**96**

Data showing recovery of endogenous LRG in a human citrate plasma sample:

	LRG [ng/ml]				Recovery [%]		
ID	**Reference**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
C1	19.0	11.2	5.3	2.6	118	111	110

Data showing recovery of endogenous LRG in a human heparin plasma sample:

	LRG [ng/ml]				Recovery [%]		
ID	**Reference**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
H1	20.5	11.7	5.8	2.8	115	113	109

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 at different locations using different ELISA lots (in-between-run precision or reproducibility).

Within-Run Precision (Intra-Assay)

Within-run precision was tested by measuring two samples of known concentrations three times within one LRG ELISA lot by one operator.

ID	n	Mean LRG [ng/ml]	SD [ng/ml]	CV [%]
Sample 1	3	3.9	0.1	2
Sample 2	3	31.7	0.8	3

In-Between-Run Precision (Inter-Assay)

In-between-run precision was tested by measuring two samples of known concentrations nine times with three kits from two different LRG ELISA lots on different days by two different operators.

ID	n	Mean LRG [ng/ml]	SD [ng/ml]	CV [%]
Sample 1	9	4.0	0.2	5
Sample 2	9	32.0	1.9	6

DETECTION LIMIT & SENSITIVITY

To determine the sensitivity of the LRG 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 from the background signal, *i.e.,* the signal that is measured in the absence of LRG, with a confidence level of 99%. It is defined as the mean back-calculated concentration of standard 1 (0 ng/ml of LRG, 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 standards containing LRG, is diluted, measured five times and its concentration back calculated. The lowest dilution, which meets both criteria, is reported as the LLOQ.

The following values were determined for the LRG ELISA:

LOD	0.26 ng/ml
LLOQ	0.5 ng/ml

SAMPLE STABILITY

Sample Collection and Storage

Serum, EDTA plasma, heparin plasma, and citrate 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.

Serum & Plasma
Collect venous blood samples in standardized blood collection tubes. Perform plasma or serum separation by centrifugation as soon as possible according to the tube manufacturer's instructions for use. Assay acquired samples immediately or aliquot and store at -25°C or lower. Lipemic or haemolyzed samples may give erroneous results. Samples are stable for at least five freeze-thaw cycles.

Freeze-Thaw Stability of Samples Containing Endogenous Human LRG

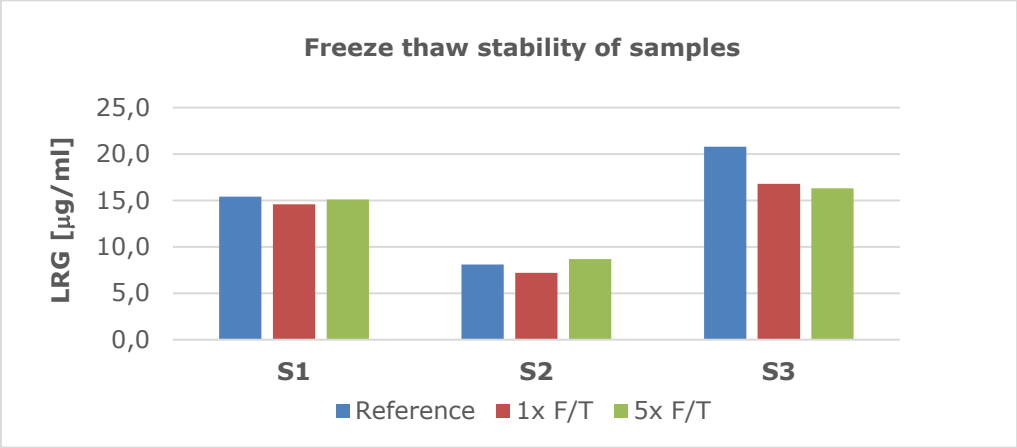
The stability of endogenous leucine-rich alpha-2-glycoprotein (LRG) was tested by comparing four measurements in samples that had undergone five freeze-thaw cycles (F/T).

For freeze-thaw experiments, samples were collected according to the supplier's instruction using blood collection devices and stored at -80°C. Reference samples were freeze-thawed once. The mean recovery of LRG in serum samples after five freeze-thaw cycles is 94%.

Leucine-rich alpha-2-glycoprotein concentrations of samples after freeze-thaw (F/T) cycles:

		LRG [µg/ml]			Recovery [%]	
Sample Matrix	**ID**	**Reference**	**1x F/T**	**5x F/T**	**1x F/T**	**5x F/T**
Serum	S1	15.4	14.6	15.1	95	98
Serum	S2	8.1	7.2	8.7	89	107
Serum	S3	20.8	16.8	16.3	81	78

All samples should undergo a maximum of five freeze-thaw cycles.



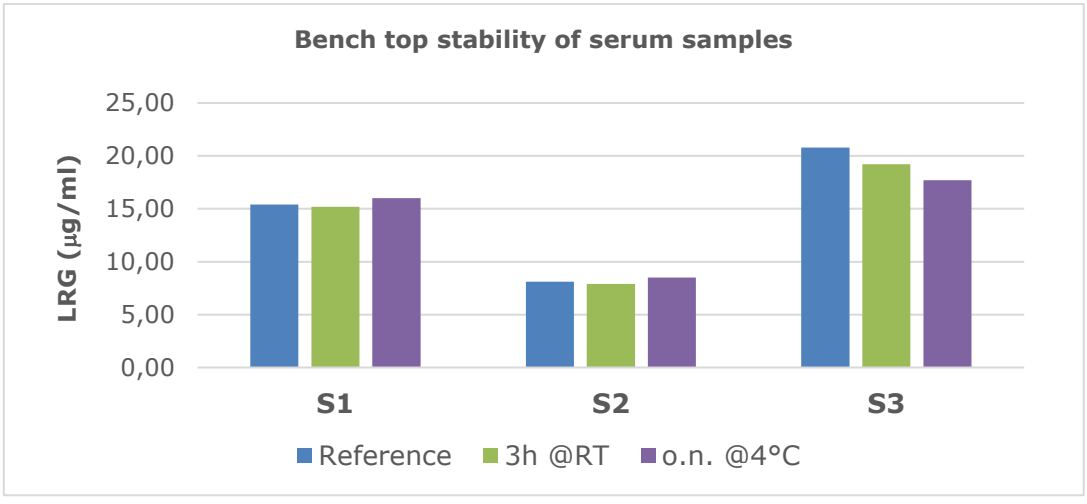
Benchtop Stability of Samples Containing Endogenous Human LRG

The benchtop stability of endogenous leucine-rich alpha-2-glycoprotein (LRG) was tested by comparing LRG measurements in human samples that had been stored at different temperatures.

For the assessment of the benchtop stability, a set of undiluted human samples was aliquoted and stored at room temperature or at 4°C. Samples can be stored for at least three hours at room temperature as well as overnight at 4°C. The mean recovery of sample concentrations after overnight storage at 4°C is 98%.

LRG concentrations of samples stored at -25°C (reference), at room temperature (RT) or overnight (o.n.) at 4°C:

		LRG (µg/ml)			Recovery [%]	
Sample Matrix	**ID**	**Reference**	**3h @RT**	**o.n. @4°C**	**3h @RT**	**o.n. @4°C**
Serum	S1	15.4	15.2	16.0	99	104
Serum	S2	8.1	7.9	8.5	98	105
Serum	S3	20.8	19.2	17.7	92	85
				Mean R [%]	**96**	**98**



SPECIFICITY

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

The specificity of the LRG ELISA was shown by characterizing both the capture and the detection antibodies through epitope mapping. In addition, the specificity of the ELISA was established through competition experiments, which measure the ability of the antibodies to exclusively bind LRG.

This assay recognizes recombinant and endogenous human LRG.

Epitope Mapping

Antibodies were characterized by epitope mapping of linear epitopes with microarray technology and by the determination of binding kinetics with biolayer interferometry. The peptide-specific coating antibody binds to a linear epitope in the N-terminal region of LRG. Multiple linear epitopes recognized by the polyclonal detection antibody are distributed over the whole LRG sequence and are located in the N- and C-terminus, as well as within the leucine-rich repeats. Both antibodies bind to LRG with low dissociation rate constants.

Competition of Signal

Competition experiments were carried out by pre-incubating human samples containing endogenous LRG with an excess of capture antibody (AB). The concentration measured in this mixture was then compared to a reference value, which was obtained from the same sample without the pre-incubation step. Mean competition in serum and plasma samples was 99%.

			LRG [ng/ml]	Competition [%]
Sample Matrix	**ID**	**Reference**	**Reference + capture AB**	
Serum	S1	5	0.1	99
Serum	S2	7	0.0	100
Serum	S3	20	0.1	99
EDTA plasma	E1	16	0.1	99
Citrate plasma	C1	15	0.1	100
Heparin plasma	H1	13	0.0	100
			Mean	**99**

Isoforms

There are no known isoforms of leucine-rich alpha-2-glycoprotein (LRG).

Cross Reactivity

The LRG sequence similarity between human LRG with mouse, rat an pig is 64%, 64% and 71%, respectively. The cross-reactivity of the human LRG ELISA with non-human samples was not tested.

CALIBRATION

The leucine-rich alpha-2-glycoprotein (LRG) ELISA kit is calibrated against recombinant human LRG protein (P02750 - Uniprot ID).

COMPARISON of the Biomedica LRG ELISA with a human LRG (leucine-rich alpha-2-glycoprotein) ELISA assay from a different manufacturer.

Assay characteristics of different human and LRG ELISA assays

	BIOMEDICA	Another MANUFACTURER
Method	Sandwich ELISA	Sandwich ELISA
Sample type	Serum, EDTA plasma, citrate plasma, heparin plasma (urine cell culture protocol available)	Plasma, serum, CSF, urine
Sample volume	100 µl pre-diluted sample / well (5 µl sample)	100 µl pre-diluted sample / well (5 µl sample)
Assay time	2 h / 1 h / 30 min	Overnight / 30 min / 30 min
Assay range	0 – 64 ng/ml (0 / 2 / 4 / 8 / 16 / 32 / 64) Assay range optimized for clinical samples, no additional testing required. Pre-dilution of samples 1:4000.	1.56 – 100 ng/ml (pre-dilution of samples 1:2000 - 1:10000)
Sensitivity	LOD: 0.26 ng/ml; LLOQ: 0.5 ng/ml	0.17 ng/ml
Specificity	Endogenous and recombinant human LRG 100%.	Human LRG 100%
Antibodies	Epitope-mapped antibodies Capture antibody: polyclonal sheep anti-human LRG antibody Detection antibody: polyclonal sheep anti-human LRG antibody, HRP-labeled Target antigen: human LRG (leucine-rich alpha-2-glycoprotein)	Capture antibody: polyclonal rabbit anti-human LRG antibody Detection antibody: polyclonal rabbit anti-human LRG antibody, HRP-labeled Target antigen: human LRG (leucine-rich alpha-2-glycoprotein)
Standard matrix	Human serum matrix containing recombinant human LRG *7 ready to use standards*	Matríx not indicated *1 stock standard vial containing recombinant human LRG*
Values of apparently healthy samples	Serum mean (n=18): 27.5 µg/ml Plasma mean (n=22): 27.9 µg/ml	Not indicated
Controls	2 controls (high and low) included	Not included
Validation	According to FDA/ICH/EMEA guidelines	Not indicated
Use	RUO	RUO

Correlation data between LRG ELISA assays (Biomedica and another manufacturer)

Measurement of LRG with the Biomedica and another LRG ELISA
in 52 samples (healthy + diseased)

Conclusion:

- Serum and plasma LRG concentrations are approximately 3-5 fold lower in the Biomedica assay than when measured with the competitor assay.
- Good correlation between both assays –
- Correlation R^2: 0.90
  Pearson correlation coefficient R = 0.85, $p < 0.00001$.

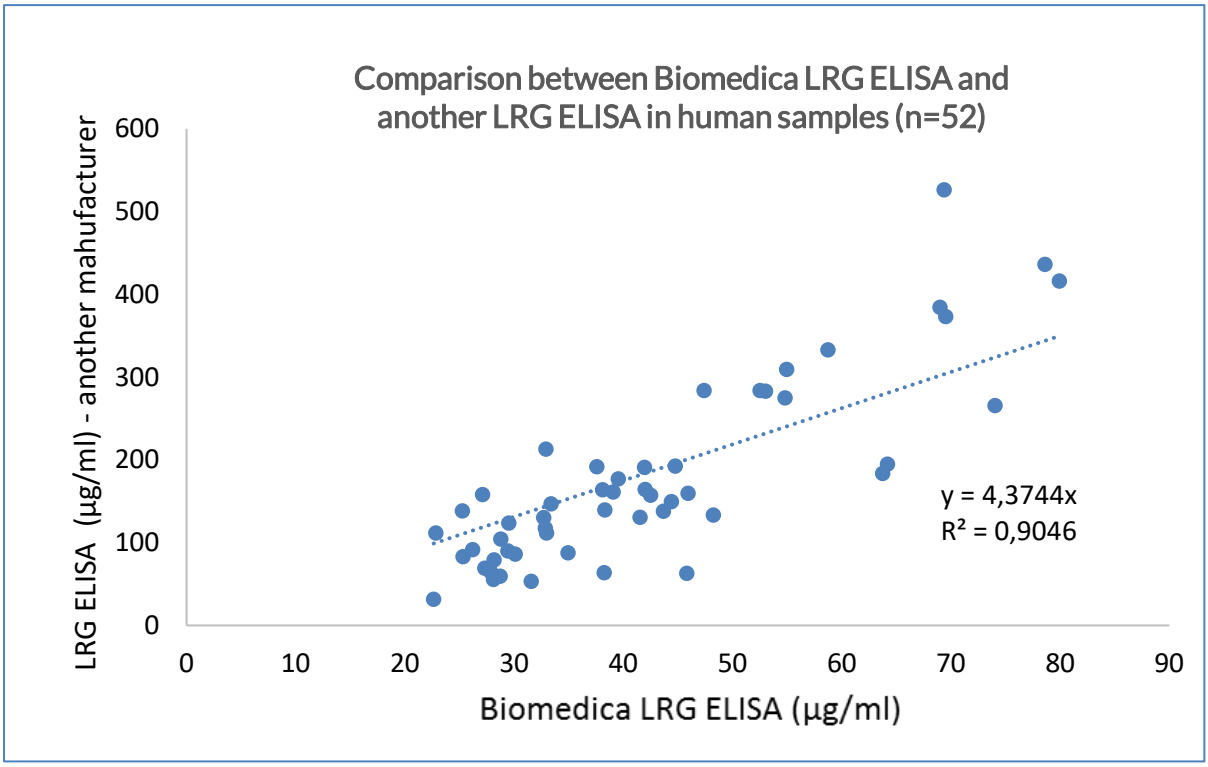


Table showing LRG concentrations measured with the Biomedica and the another LRG ELISA assay:

	ALL SAMPLES		
	n = 52		
	Sample ID	Biomedica	other
Cohorts:		LRG, µg/ml	LRG, µg/ml
Apparently healhty (AH) samples	AH1	33	147
	AH2	29	104
	AH3	25	138
	AH4	33	130
	AH5	33	213
	AH6	23	112
	AH7	27	158
	AH8	25	83
	AH9	33	112
	AH10	28	65
	AH11	29	90
Nephrology (N) samples	N12	43	157
	N13	64	183
	N14	79	436
	N15	40	177
	N16	46	159

	N17	42	191
	N18	55	275
	N19	44	150
Unspecific hospital panel (UHP) samples	UHP20	46	63
	UHP21	38	139
	UHP22	42	131
	UHP23	69	526
	UHP24	28	56
	UHP25	38	63
	UHP26	23	31
	UHP27	28	79
	UHP28	64	195
	UHP29	32	53
	UHP30	80	416
Cardiology (C) samples	C31	33	117
	C32	30	124
	C33	44	138
	C34	29	59
	C35	74	266
	C36	27	69
	C37	48	133
	C38	55	309
	C39	47	284
	C40	69	384
	C41	38	164
	C42	35	88
	C43	42	164
	C44	30	86
	C45	26	91
Rheuma (R) samples	R46	59	333
	R47	53	284
	R48	70	373
	R49	39	161
	R50	38	192
	R51	53	283
	R52	45	192
		Pearson	**0.85**
		p value	< 0.00001

Performance Check on the measurement of LRG in human urine samples

Accuracy – Urine Samples

The recovery of human urine samples in the LRG ELISA was tested by adding recombinant LRG to urine samples containing a known concentration of endogenous LRG. The %recovery of the spiked concentration was calculated as the percentage of measured over the expected value.

This table shows the summary of the recovery experiments in the LRG ELISA in urine samples:

URINE SAMPLES - accuracy - samples spiked with recombinant LRG:+32ng/ml - 1+1; +16ng/ml - 1+1; +8ng/ml - 7+1

Sample Matrix	ID	dilution	LRG1 c[ng/ml]				% Recovery		
			Reference	+32 ng/ml	+16 ng/ml	+8,0 ng/ml	+32 ng/ml	+16 ng/ml	+8,0 ng/ml
urine	healthy U2	01:10	>70,400	63,13	51,69	65,22			
urine	healthy U3	01:10	0,52	32,46	16,16	8,93	101%	99%	106%
urine	healthy U4	01:20	3,23	34,87	17,25	11,02	104%	98%	102%
urine	healthy U8	01:20	1,63	32,82	16,64	9,48	100%	99%	101%
urine	nephro 74	1:3000	11,76	37,10	22,04	18,25	98%	101%	100%
urine	nephro 86	1:2000	2,56	32,37	17,30	10,10	97%	100%	98%
urine	nephro 68	1:500	19,52	41,43	24,40	25,25	99%	92%	102%
urine	nephro 80	1:100	43,46	51,85	38,99	46,11	94%	108%	101%
						Mean	**99%**	**100%**	**101%**
						min	94%	92%	98%
						max	104%	108%	106%

Sample Matrix	ID	dilution	LRG1 c[ng/ml]				% Recovery		
			Reference	+32 ng/ml	+16 ng/ml	+8,0 ng/ml	+32 ng/ml	+16 ng/ml	+8,0 ng/ml
urine	healthy U8	1:2	9,31	41,12		15,84	114%		96%
urine		1:5	4,30	39,65		12,27	117%		106%
urine		1:10	1,97	38,20		11,56	116%		123%
urine		1:20	1,13	39,43		10,54	121%		119%
urine	healthy U3	1:2	26,59	51,25		30,74	119%		93%
urine		1:4	14,55	42,83		18,85	111%		76%
urine		1:8	9,42	45,75		16,53	128%		104%
						Mean	**118%**		**103%**
						min	111%		76%
						max	128%		123%

DILUTION LINEARITY & PARALLELISM

Dilution Linearity – Urine Samples

Experiment:
Dilution linearity was assessed by serially diluting samples containing **recombinant** LRG with assay buffer.

The table below shows the mean recovery and range of serially diluted recombinant LRG in human urine samples.

URINE SAMPLES - dilution linearity

dilution of the samples spiked 1+1 with recombinant human LRG (+32ng/ml) - in assay buffer - 1+1, 1+3

				LRG1 c[ng/ml]		% Recovery	
Sample Matri	ID	dilution	ref	1+1	1+3	1+1	1+3
urine	healthy U2	01:10	63,13	28,91	13,69	92%	87%
urine	healthy U3	01:10	32,46	14,54	6,95	90%	86%
urine	healthy U4	01:20	34,87	14,93	7,36	86%	84%
urine	healthy U8	01:20	32,82	14,79	7,05	90%	86%
urine	nephro 74	1:3000	37,10	16,59	8,71	89%	94%
urine	nephro 86	1:2000	32,37	14,79	7,74	91%	96%
urine	nephro 68	1:500	41,43	18,34	8,96	89%	87%
urine	nephro 80	1:100	51,85	23,06	11,46	89%	88%
					Mean	**89%**	**88%**
					min	86%	84%
					max	92%	96%

Parallelism – Urine Samples

Experiment:
Parallelism was assessed by serially diluting human samples containing **endogenous** LRG with assay buffer.

The table below shows the summary of the mean recovery and range of serially diluted endogenous human LRG in human urine samples.

URINE SAMPLES - parallelism dilution of urine samples containing endogenous LRG in assay buffer - 1+1, 1+3, 1+7

			LRG1 c[ng/ml]				% Recovery		
Sample Matrix	ID	dilution	Reference	1+1	1+3	1+7	1+1	1+3	1+7
urine	healthy U2	01:10	>70,400	41,22	16,82				
urine	healthy U4	01:20	3,23	1,37	0,78		85%	97%	
urine	nephro 74	1:3000	11,76	4,88	2,39	1,22	83%	81%	83%
urine	nephro 68	1:500	19,52	7,66	3,77	1,95	78%	77%	80%
urine	nephro 80	1:100	43,46	18,65	9,32	4,55	86%	86%	84%
						Mean	**83%**	**85%**	**82%**

			LRG1 c[ng/ml]				% Recovery		
Sample Matrix	ID	dilution	Reference	1+1	1+3	1+7	1+1	1+3	
urine	healthy U5R	1:2	43,59	26,07	14,13		120%	130%	
urine	healthy U1-2	1:2	61,96	31,70	17,23		102%	111%	
urine	healthy U2-2	1:2	17,23	9,04	4,81		105%	112%	
urine	healthy U7-2	1:2	29,94	15,23	8,13		102%	109%	
urine	healthy U3-2	1:2	7,70	3,66	1,69		95%	88%	
urine	healthy U6-2	1:2	4,30	2,18	0,98		101%	91%	
						Mean	**104%**	**107%**	
						min	95%	88%	
						max	120%	130%	

REFERENCES & DOCUMENTS

Validation Literature

The assay is fully validated according to:

1. ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology”
2. EMEA/CHMP/EWP/192217/2009 Guideline on bioanalytical method validation
3. Bioanalytical Method Validation, Guidance for Industry, FDA, May 2018

Additional Documents Available Online (www.bmgrp.com)

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