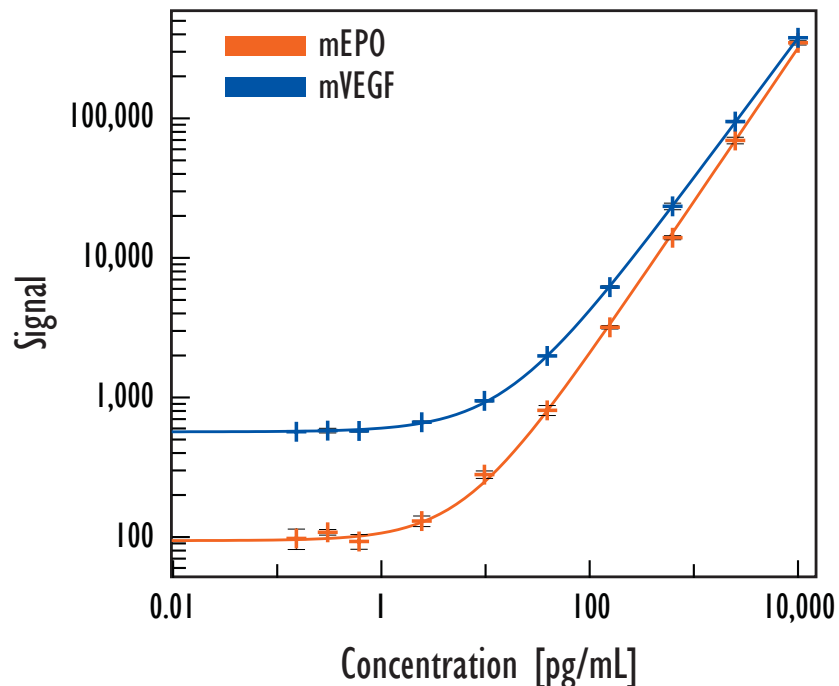


# MULTI-SPOT<sup>®</sup> Mouse/Rat Hypoxia Serum/Plasma Kit

## Assay Performance for Mouse Serum and Plasma Samples



Concentration (pg/mL)	mEPO		mVEGF	
	Mean	%CV	Mean	%CV
0	89	11	568	5
0.2	98	17	568	1
0.3	108	5	579	4
0.6	93	12	576	2
2	130	9	665	2
10	280	6	947	2
39	809	8	1,986	1
156	3,187	3	6,182	2
625	13,959	3	23,424	5
2,500	69,469	5	94,919	2
10,000	347,604	3	377,911	1

Standard curve data is from a representative experiment.

Detection Limit (pg/mL)	mEPO	mVEGF
Average LLOD	2.3	1.8

LLOD is defined as 2.5x stdev above the background.

### Endogenous Levels in Mouse Samples

	pg/mL	mEPO	mVEGF
Plasma	Mean	425	10
	Median	321	10
	Range	40 - 2285	8 - 14
Serum	Mean	122	15
	Median	107	15
	Range	56 - 246	12 - 18

- Heparin treated plasma samples were obtained from 5 male and 4 female individuals. Serum samples were obtained from 5 male and 5 female individuals.
- Detected level was above LLOQ for all analytes in all samples.
- Average CVs for measured samples was less than 12%.

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## Mouse — Linearity of Dilution and Spike Recovery

### Dilution Linearity

- Measured analyte levels in samples diluted into assay diluent at different dilution factors prior to measurement in an assay
- Dilution Linearity was determined from both unspiked and spiked (1 ng/mL) pooled samples

$$\% \text{ recovery} = \frac{(\text{measured value} * \text{dilution factor} * 100)}{\text{predicted value}}$$

### Unspiked Sample Dilution Linearity

	Dilution Factor	mEPO	mVEGF
Serum	3/4	97	97
	1/2	98	102
	1/4	98	99
EDTA Plasma	3/4	101	93
	1/2	103	93
	1/4	114	100
Heparin Plasma	3/4	109	102
	1/2	114	104
	1/4	124	118

### Spiked Sample Dilution Linearity

	Dilution Factor	mEPO	mVEGF
Serum	3/4	95	102
	1/2	97	109
	1/4	94	114
EDTA Plasma	3/4	98	103
	1/2	105	108
	1/4	110	113
Heparin Plasma	3/4	94	94
	1/2	97	101
	1/4	97	98

Values presented are averages across 4 pools of serum, 3 pools of EDTA plasma, 2 pools of Heparin plasma.

### Spike Recovery

- Measured analyte spiked into undiluted mouse samples

$$\% \text{ recovery} = \frac{(\text{measured value} * 100)}{\text{expected value}}$$

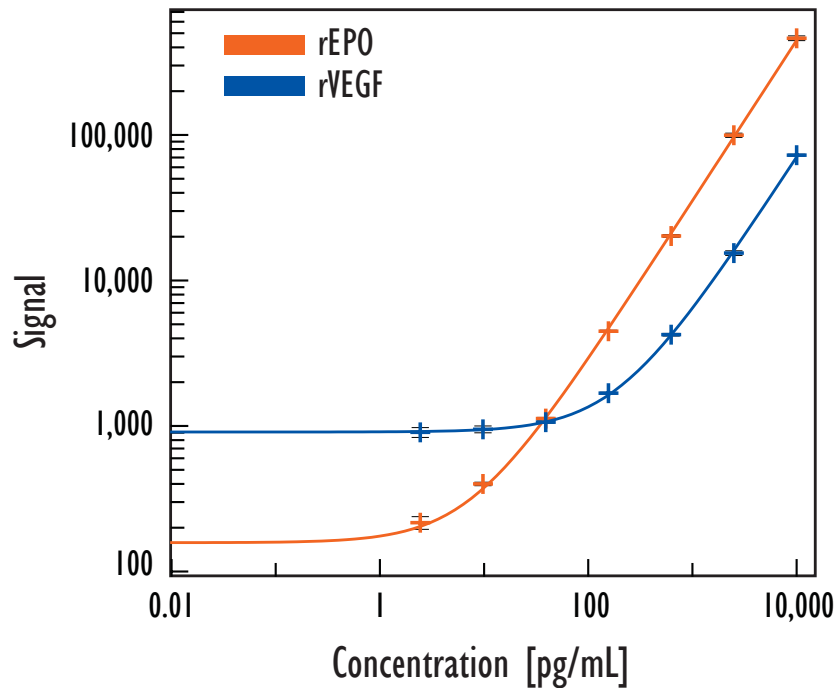
	mEPO	mVEGF
Serum	95	58
EDTA Plasma	87	70
Heparin Plasma	93	81

Values presented are averages across 4 pools of serum, 3 pools of EDTA plasma, 2 pools of Heparin plasma and including spikes of 1000, 500 and 200 pg/ml. Spike recovery of mouse VEGF was low, presumably because binding proteins such as soluble VEGF receptors bind to some the spiked VEGF.

Kit Size	SI2400	SI6000
1 plate	K15123C-1	K11123C-1
5 plates	K15123C-2	K11123C-2
20 plates	K15123C-3	K11123C-3
20 plates (Base)	K15123A-3	K11123A-3

# MULTI-SPOT<sup>®</sup> Mouse/Rat Hypoxia Serum/Plasma Kit

## Assay Performance for Rat Serum and Plasma Samples



Concentration (pg/mL)	rEPO		rVEGF	
	Mean	%CV	Mean	%CV
0	149	5	906	4
2	217	10	905	8
10	400	3	949	5
39	1,125	2	1,063	2
156	4,482	2	1,682	1
625	20,229	2	4,243	2
2,500	99,561	3	15,428	4
10,000	462,163	4	72,620	1

Standard curve data is from a representative experiment.

Detection Limit (pg/mL)	rEPO	rVEGF
Average LLOD	2	19

LLOD is defined as 2.5x stdev above the background.

### Endogenous Levels in Rat Samples (pg/mL)

	rEPO	rVEGF
Serum	63	258
EDTA Plasma	57	161
Heparin Plasma	86	227

- Serum values were obtained from the average of 2 rat serum pools each pool containing serum from 10 individual animals.
- Heparin plasma values were obtained from one rat Heparin plasma pool containing 10 individual animals.
- EDTA plasma values were obtained from one rat EDTA plasma pool containing 10 individual animals.

# MULTI-SPOT<sup>®</sup> Mouse/Rat Hypoxia Serum/Plasma Kit

## Rat — Linearity of Dilution and Spike Recovery

### Dilution Linearity

- Measured analyte levels in samples diluted to 50% into assay diluent prior to measurement in an assay

$$\% \text{ recovery} = \frac{(\text{measured value} * \text{dilution factor} * 100)}{\text{predicted value}}$$

### Unspiked Sample Dilution Linearity

	Dilution Factor	rEPO	rVEGF
Serum	1/2	125	113
EDTA Plasma	1/2	106	100
Heparin Plasma	1/2	113	98

Values presented are averages across 2 pools of serum, 1 pool of EDTA plasma, 1 pool of Heparin plasma. Each pool represents 20 individuals.

### Spike Recovery

- Measured analyte spiked into undiluted rat samples

$$\% \text{ recovery} = \frac{(\text{measured value} * 100)}{\text{expected value}}$$

	rEPO	rVEGF
Serum	94	59
EDTA Plasma	96	65
Heparin Plasma	96	53

Values presented are from pooled samples. Each pool represents 20 individuals. Values are obtained from 2 pools of serum, 1 pool of EDTA plasma, and 1 pool of Heparin plasma, including spikes of 1000 and 500 pg/ml. Spike recovery of rat VEGF was low, presumably because binding proteins such as soluble VEGF receptors bind to some the spiked VEGF.

Kit Size	SI2400	SI6000
1 plate	K15123C-1	K11123C-1
5 plates	K15123C-2	K11123C-2
20 plates	K15123C-3	K11123C-3
20 plates (Base)	K15123A-3	K11123A-3