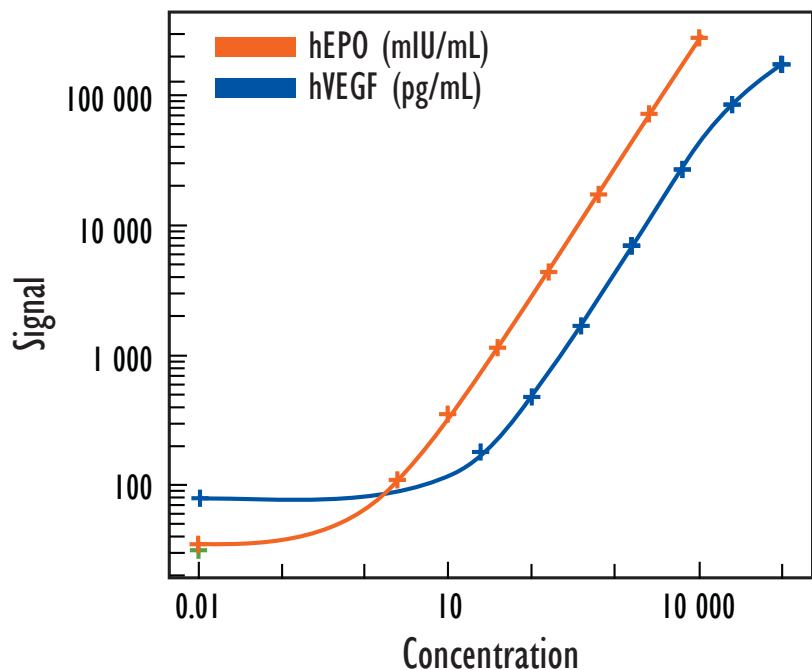


MULTI-SPOT[®] Human Hypoxia Serum/Plasma Assay

Detection of Human Erythropoietin (EPO) and Vascular Endothelial Growth Factor (VEGF) in Serum and Plasma



Concentration (mIU/mL)	hEPO		Concentration (pg/mL)	hVEGF	
	Mean	%CV		Mean	%CV
0	35	5.7	0	79	7.0
2	110	6.8	24	180	5.3
10	351	9.2	98	474	1.5
39	1 162	4.2	391	1 680	5.1
156	4 336	5.0	1 563	7 066	1.9
625	17 279	3.9	6 250	27 148	2.4
2 500	72 429	0.8	25 000	85 195	6.5
10 000	278 806	2.4	100 000	173 162	2.5

Above is representative calibration curve data.

	hEPO	hVEGF
Average LLOD	1.1 mIU/mL	12 pg/mL

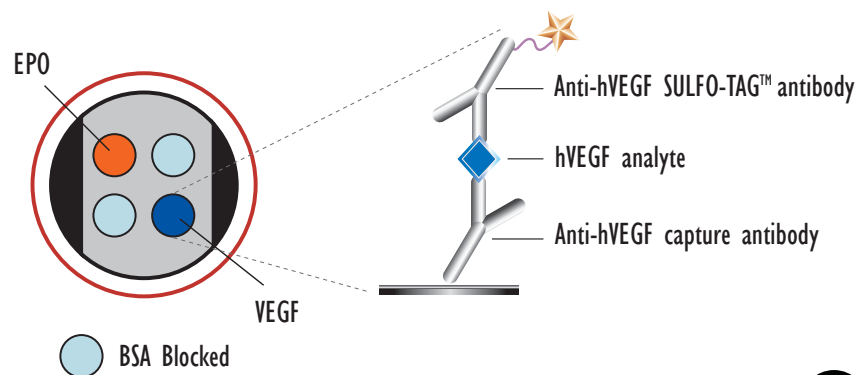
LLOD is defined as 2.5x stdev above the background.

Kit Size	Catalog Number
1 plate	K15122C-1
5 plates	K15122C-2
20 plates	K15122C-3
20 plates (Base)	K15122A-3

Endogenous Levels

		hEPO (mIU/mL)	hVEGF (pg/mL)
Serum	Min	9	n/d
	Max	40	78
	Median	14	49
EDTA Plasma	Min	6	n/d
	Max	26	940
	Median	9	65
Heparin Plasma	Min	5	33
	Max	27	374
	Median	8	73

Normal human samples were measured using 8 sera, EDTA plasma, and heparin plasma. If measured values are below the LLOD, they are indicated as n/d.



MULTI-SPOT Human Hypoxia Serum/Plasma Assay

Linearity of Dilution and Spike Recovery

Dilution Linearity

- Three pools each of human serum and human heparin plasma were evaluated; a representative pool of each is shown. The pooled samples were spiked at mid level with calibrator and then diluted with Assay Diluent GFI. The concentrations shown have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration for the previous dilution (expected).

$$\% \text{ Recovery} = (\text{measured} \times \text{dilution factor}) / \text{expected} \times 100$$

	Fold Dilution	hEPO (mIU/mL)			hVEGF (pg/mL)		
		Conc. (mIU/mL)	% CV	% Recovery	Conc. (pg/mL)	% CV	% Recovery
Spiked Serum	Neat	1382	3		13733	2	
	4	1301	3	94	12733	2	93
	16	1059	0	81	11043	1	87
	64	1090	12	103	9730	12	88
Spiked Plasma	Neat	1136	9		11384	3	
	4	1219	2	103	12152	6	111
	16	844	6	80	9822	3	87
	64	772	22	94	9570	19	102

Spike Recovery

- Human serum and heparin plasma were spiked with calibrators at multiple levels throughout the range of the assay. Each spike was done in ≥ 3 replicates. The Calibrators were spiked into individual samples and therefore spike recovery may depend on specific individual samples.

$$\% \text{ Recovery} = \text{measured} / \text{expected} \times 100$$

hEPO	Spike Level (mIU/mL)	Expected Conc. (mIU/mL)	Measured Conc. (mIU/mL)	% CV	% Recovery
Spiked Serum	0	12	12	15	
	45	57	53	6	92
	213	226	222	7	98
	1136	1149	1089	7	95
Spiked Heparin Plasma	0	36	36	17	
	45	81	81	7	101
	213	249	255	7	102
	1136	1172	1222	7	104

hVEGF	Spike Level (pg/mL)	Expected Conc. (pg/mL)	Measured Conc. (pg/mL)	% CV	% Recovery
Spiked Serum	0	21	21	18	
	422	443	481	3	109
	2084	2096	2307	5	110
	12014	12026	12518	4	104
Spiked Heparin Plasma	0	24	24	21	
	422	446	462	3	103
	2084	2108	2309	4	110
	12014	12038	12708	3	106