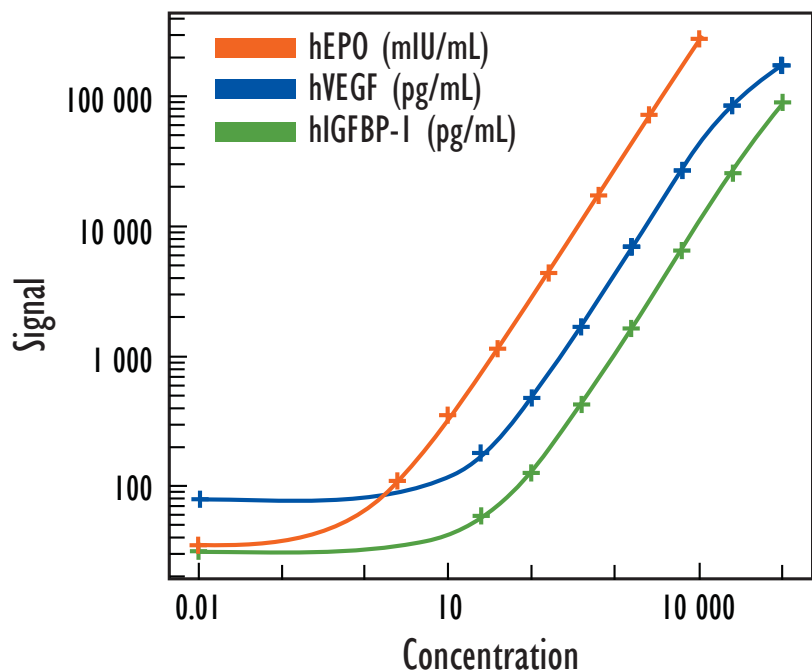


# MULTI-SPOT<sup>®</sup> Human Hypoxia Serum/Plasma Assay

Detection of Human Erythropoietin (EPO), Insulin-like Growth Factor Binding Protein -1 (IGFBP-1) and Vascular Endothelial Growth Factor (VEGF) in Serum and Plasma



| Concentration (mIU/mL) | hEPO   |     | Concentration (pg/mL) | hVEGF  |     | Concentration (pg/mL) | hIGFBP-1 |      |
|------------------------|--------|-----|-----------------------|--------|-----|-----------------------|----------|------|
|                        | Mean   | %CV |                       | Mean   | %CV |                       | Mean     | %CV  |
| 0                      | 35     | 5.7 | 0                     | 79     | 7.0 | 0                     | 31       | 22.6 |
| 2                      | 110    | 6.8 | 24                    | 180    | 5.3 | 24                    | 61       | 2.5  |
| 10                     | 351    | 9.2 | 98                    | 474    | 1.5 | 98                    | 126      | 6.6  |
| 39                     | 1162   | 4.2 | 391                   | 1680   | 5.1 | 391                   | 426      | 7.2  |
| 156                    | 4336   | 5.0 | 1563                  | 7066   | 1.9 | 1563                  | 1646     | 3.5  |
| 625                    | 17279  | 3.9 | 6250                  | 27148  | 2.4 | 6250                  | 6430     | 1.8  |
| 2500                   | 72429  | 0.8 | 25000                 | 85195  | 6.5 | 25000                 | 26217    | 3.2  |
| 10000                  | 278806 | 2.4 | 100000                | 173162 | 2.5 | 100000                | 89316    | 5.9  |

Above is representative calibration curve data.

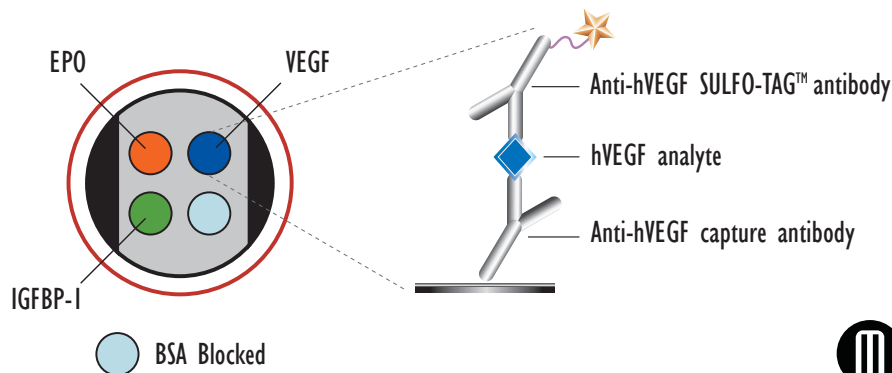
|              | hEPO       | hVEGF    | hIGFBP-1 |
|--------------|------------|----------|----------|
| Average LLOD | 1.1 mIU/mL | 12 pg/mL | 27 pg/mL |

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) | hIGFBP-1 (pg/mL) |
|----------------|--------|---------------|---------------|------------------|
| Serum          | Min    | 9             | n/d           | 208              |
|                | Max    | 40            | 78            | 8980             |
|                | Median | 14            | 49            | 548              |
| EDTA Plasma    | Min    | 6             | n/d           | 301              |
|                | Max    | 26            | 940           | 6374             |
|                | Median | 9             | 65            | 719              |
| Heparin Plasma | Min    | 5             | 33            | 151              |
|                | Max    | 27            | 374           | 4039             |
|                | Median | 8             | 73            | 506              |



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 GF1. 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) |      |            | hIGFBP-1 (pg/mL) |      |            |
|---------------|---------------|----------------|------|------------|---------------|------|------------|------------------|------|------------|
|               |               | Conc. (mIU/mL) | % CV | % Recovery | Conc. (pg/mL) | % CV | % Recovery | Conc. (pg/mL)    | % CV | % Recovery |
| Spiked Serum  | Neat          | 1382           | 3    |            | 13733         | 2    |            | 8793             | 7    |            |
|               | 4             | 1301           | 3    | 94         | 12733         | 2    | 93         | 9263             | 6    | 105        |
|               | 16            | 1059           | 0    | 81         | 11043         | 1    | 87         | 9777             | 4    | 106        |
|               | 64            | 1090           | 12   | 103        | 9730          | 12   | 88         | 10918            | 4    | 112        |
| Spiked Plasma | Neat          | 1136           | 9    |            | 11384         | 3    |            | 8295             | 10   |            |
|               | 4             | 1219           | 2    | 103        | 12152         | 6    | 111        | 8910             | 5    | 118        |
|               | 16            | 844            | 6    | 80         | 9822          | 3    | 87         | 8685             | 9    | 112        |
|               | 64            | 772            | 22   | 94         | 9570          | 19   | 102        | 10210            | 17   | 113        |

### 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  $\geq 3$  replicates. The Calibrators were spiked into individual samples and therefore spike recovery may depend on specific individual samples.

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

| 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        |

| 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        |

| hIGFBP-1              | Spike Level (pg/mL) | Expected Conc. (pg/mL) | Measured Conc. (pg/mL) | % CV | % Recovery |
|-----------------------|---------------------|------------------------|------------------------|------|------------|
| Spiked Serum          | 0                   | 408                    | 408                    | 9    |            |
|                       | 561                 | 970                    | 797                    | 4    | 82         |
|                       | 2466                | 2874                   | 2315                   | 6    | 81         |
|                       | 11849               | 12257                  | 9277                   | 4    | 76         |
| Spiked Heparin Plasma | 0                   | 906                    | 906                    | 5    |            |
|                       | 561                 | 1467                   | 1293                   | 5    | 88         |
|                       | 2466                | 3372                   | 3087                   | 5    | 92         |
|                       | 11849               | 12754                  | 11504                  | 6    | 90         |