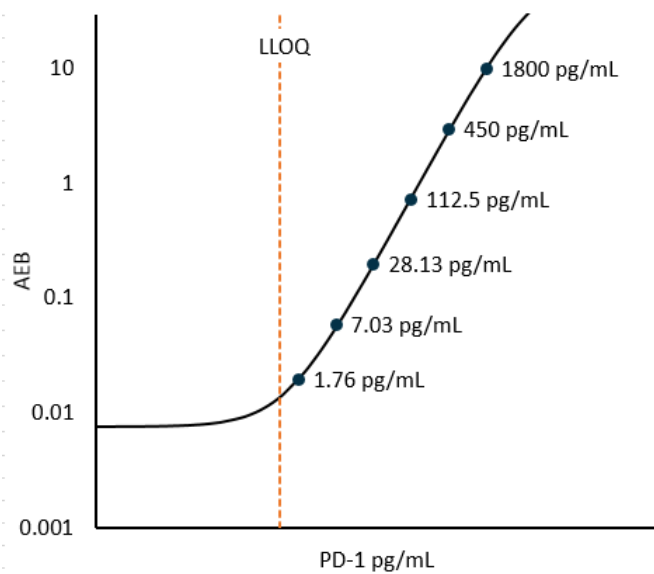


**Description**

Programmed cell death protein 1 (PD-1 or CD279) is a cell surface receptor that belongs to the immunoglobulin superfamily and is expressed on T cells, B cells, monocytes, and dendritic cells. PD-1 plays an important role as an immune checkpoint. PD-1 binds to two ligands, PD-L1 and PD-L2. The PD-1/PD-L1 or PD-L2 signaling pathway is a negative regulatory mechanism that inhibits T cell proliferation and cytokine production<sup>1</sup>. PD-1 inhibitors play a role in activation of the immune system and can be used for cancer treatment. Blockade of the PD-1/PD-L1 interaction enhances anti-tumor immunity and shows potential for improving cancer immunotherapy<sup>2</sup>. The PD-1 pathway plays a major role in the inhibition of self-reactive T cells and protection against autoimmune diseases<sup>3,4</sup>. Rheumatoid arthritis patients were shown to have significantly elevated plasma levels of sPD-1<sup>5</sup>. Serum sPD-1 levels positively correlated with the severity of skin sclerosis<sup>6</sup>. Autoimmune hepatitis patients with active disease and incomplete response to standard treatment showed increased sPD-1 levels<sup>7</sup>. PD-1 was also shown to be a regulator of virus-specific CD8+ T cell survival in HIV infection<sup>8</sup>.

**Calibration Curve:** Calibrator concentrations and Lower Limit of Quantification depicted.



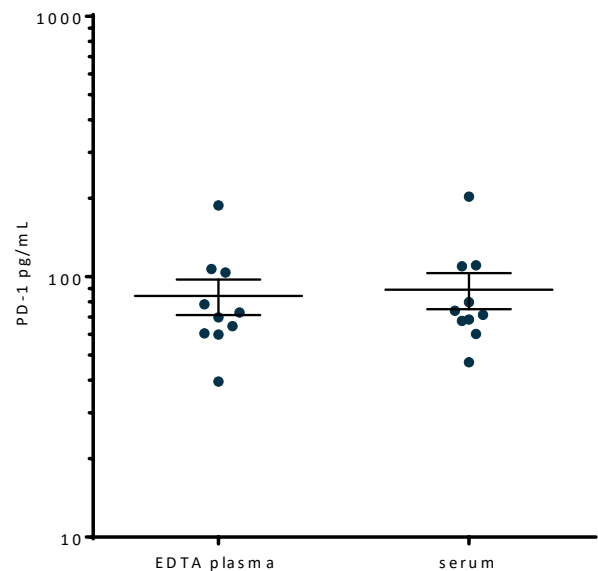
**Lower Limit of Quantification (LLOQ):** Triplicate measurements of serially diluted calibrator were read back on the calibration curve for 5 runs each for 1 reagent lot on a single instrument (5 runs total). The LLOQ is determined as the lowest dilution with a pooled CV ≤ 20% and sample concentration recovery between 80-120% of the expected.

**Limit of Detection (LOD):** Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration over 5 runs for 1 reagent lot on a single instrument (5 runs total).

<b>LLOQ</b>	<b>0.879 pg/mL</b> pooled CV 19%, mean recovery 98%
<b>LOD</b>	<b>0.247 pg/mL</b> range 0.109-0.370 pg/mL
<b>Sample range</b>	0–7200 pg/mL
<b>Diluted sample volume*</b>	100 µL Per measurement
<b>Tests per kit</b>	192

**\*See Kit Instruction for details**

**Endogenous Sample Reading:** Healthy donor matched EDTA plasma (n=10) and serum (n=10) samples were measured. Bars depict mean with SEM.



Matched human samples (n=10)	Mean PD-1 pg/mL	Median PD-1 pg/mL	% Above LOD
EDTA plasma	84.4	71.3	100%
Serum	89.2	72.7	100%

**Precision on HD-1:** Measurements of 3 serum or plasma based panels. Triplicate measurements were made for 5 runs using 1 reagent lot and a single instrument (5 runs total, 15 measurements).

Sample	Mean (pg/mL)	Within run CV	Between run CV
Panel 1	158	2.8%	13.4%
Panel 2	924	5.1%	5.3%
Panel 3	480	2.3%	7.8%

**Spike and Recovery:** 2 EDTA plasma samples and 2 serum samples were spiked at high and low concentrations within the range of the assay.

**Dilution Linearity:** 1 spiked endogenous EDTA plasma sample and 1 spiked endogenous serum sample were diluted 2x serially from MRD (4x) to 256x with Sample Diluent.

<b>Spike and Recovery (Serum/Plasma)</b>	<b>108%</b> Range 89-132%
<b>Spiked Plasma Dilution Linearity (256x)</b>	<b>Mean = 106%</b> Range: 99-113%
<b>Spiked Serum Dilution Linearity (256x)</b>	<b>Mean = 121%</b> Range: 114-130%

**Specificity:** Normal serum (n=2) and EDTA plasma (n=2) were directly incubated with detector antibody and run at MRD. Average knock-down was **96.8%** with a range of **96.5% -97.1%**.

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