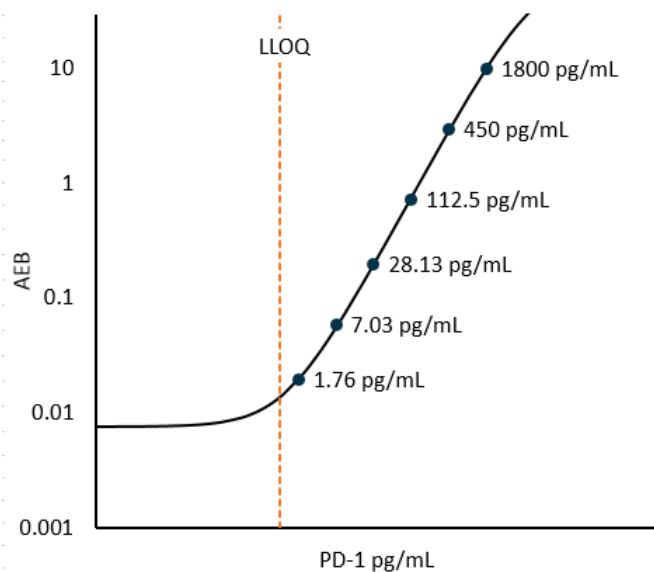


Description

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 production¹. 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 immunotherapy². The PD-1 pathway plays a major role in the inhibition of self-reactive T cells and protection against autoimmune diseases^{3,4}. Rheumatoid arthritis patients were shown to have significantly elevated plasma levels of sPD-1⁵. Serum sPD-1 levels positively correlated with the severity of skin sclerosis⁶. Autoimmune hepatitis patients with active disease and incomplete response to standard treatment showed increased sPD-1 levels⁷. PD-1 was also shown to be a regulator of virus-specific CD8+ T cell survival in HIV infection⁸.

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



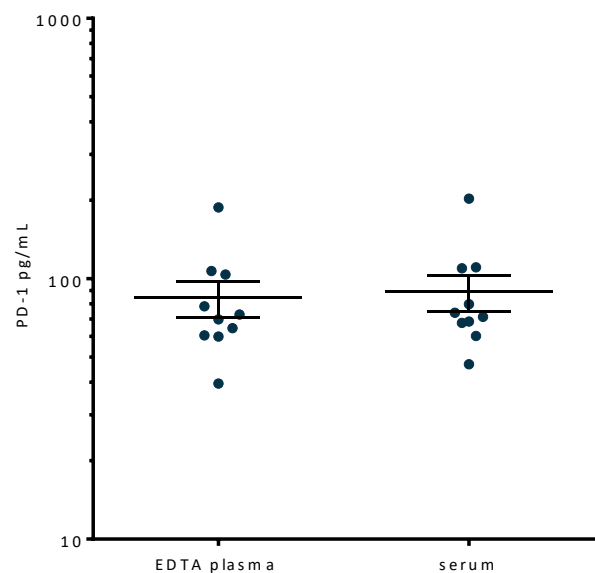
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve for 5 runs each for 1 reagent lot on a single instrument (5 runs total). The LLOQ is determined as the lowest dilution with a pooled CV \leq 20% and sample concentration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

| | |
|-------------------------------|---|
| LLOQ | 0.879 pg/mL pooled CV 19%, mean recovery 98% |
| LOD | 0.247 pg/mL range 0.109-0.370 pg/mL |
| Sample range | 0–7200 pg/mL |
| Diluted sample volume* | 100 μ L Per measurement |
| Tests per kit | 96 |

***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.

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