**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. 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.

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

**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).

| LLOQ                        | 0.879 pg/mL  
|-----------------------------|--------------
| pooled CV                   | 19%, mean recovery 98% |
| LOD                         | 0.247 pg/mL  
| Sample range                | range 0.109-0.370 pg/mL |
| Diluted sample volume*      | 100 μL       
| 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.
**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).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/mL)</th>
<th>Within run CV</th>
<th>Between run CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel 1</td>
<td>158</td>
<td>2.8%</td>
<td>13.4%</td>
</tr>
<tr>
<td>Panel 2</td>
<td>924</td>
<td>5.1%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Panel 3</td>
<td>480</td>
<td>2.3%</td>
<td>7.8%</td>
</tr>
</tbody>
</table>

**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.

**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%.

**References:**