Signal transducer and activator of transcription 4 (STAT4) is a transcription factor that transduces interleukin-12, interleukin-23, and type-1 interferon cytokine signals in T-cells and monocytes. Following exposure to cytokines, the cytokine receptor-associated Janus kinases (JAK) phosphorylate tyrosine residues present on cytoplasmic STAT4 proteins. STAT4 phosphorylation at tyrosine residue 693 allows homodimerization through src homology 2 domains. Functional STAT4 dimers translocate into the nucleus and activate cytokine responsive gene transcription, leading to Th1 cell differentiation, monocyte activation, and interferon-gamma production. STAT4 contributes to autoimmune disorder pathogenesis and anti-viral immune responses.

The MSD Total STAT4 assay is available on 96-well, 4-spot plates. This datasheet outlines the performance of the assay.

**Typical Data**

Representative results for the Total STAT4 Kit are illustrated below. The signal and ratio values provided are examples; individual results will vary depending upon the samples tested. Western blot analyses of each lysate type are shown for comparison.

Growing human T cells were starved for 30 minutes (treatment 1) or starved for 30 minutes then co-incubated with IL-12 (10 ng/mL) and interferon-alpha (1000 U/mL) for 30 minutes (treatment 2). Whole cell lysates were added to MSD MULTI-SPOT®, 4-spot plates coated with anti-total STAT4 antibody on one of the four spatially distinct electrodes in each well. Phospho-STAT4 (Tyr693) was detected with anti-total STAT4 antibody conjugated with MSD SULFO-TAG™ reagent.

**Figure 1:** Sample data generated with Total STAT4 assay. Increased signal is observed with the titration of lysates starved for 30 minutes (treatment 1) and with lysates starved for 30 minutes then co-incubated with IL-12 (10 ng/mL) and interferon-alpha (1000 U/mL) for 30 minutes (treatment 2). The Total STAT4 assay provides a quantitative measure of the data obtained with the traditional Western blot.
Lysate Titration

Data for positive and negative cell lysates using the Total STAT4 Kit are presented below.

<table>
<thead>
<tr>
<th>Lysate (µg/well)</th>
<th>Treatment 1</th>
<th></th>
<th></th>
<th>Treatment 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average Signal</td>
<td>StdDev</td>
<td>%CV</td>
<td>Average Signal</td>
<td>StdDev</td>
<td>%CV</td>
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<tr>
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<td>2713</td>
<td>1.7</td>
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<td>291 583</td>
<td>6706</td>
<td>2.3</td>
<td>568 487</td>
<td>7959</td>
<td>1.4</td>
</tr>
</tbody>
</table>

For a complete list of products, please visit our website at www.mesoscale.com.

The MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample volumes of 25 µL or less without compromising speed or performance
- **Large dynamic range:** Linear range of up to five logs enables the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions
- **Minimal background:** The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference
- **Simple protocols:** Only labels bound near the electrode surface are excited, enabling assays with fewer washes
- **Flexibility:** Labels are stable, non-radioactive, and conveniently conjugated to biological molecules

References