**ERK** (Extracellular signal Regulated Kinases) 1 and 2, are proline-directed serine/threonine protein kinases, also known as p44 MAPK (Mitogen-Activated Protein Kinase) and p42 MAPK, respectively. These closely-related kinase isoforms are activated by various extracellular signals including growth factors, cytokines, hormones, and neurotransmitters. The activation occurs through the phosphorylation of threonine 202 and tyrosine 204 of ERK1 and threonine 185 and tyrosine 187 of ERK2 by the upstream kinases MEK1 and MEK2. Activated ERK1/2 phosphorylates targets in both the cytosol and the nucleus. Cytosolic substrates for ERK include SOS, MNK1/2, and the 90 kDa ribosomal protein S6 kinases (RSKs). Nuclear translocation of activated ERK affects gene expression and DNA replication by the phosphorylation of MSK 1 and 2 and the transcription factors Elk-1, Sap1, and Sap2.

The MSD Phospho(Thr202/Tyr204; Thr185/Tyr187)/Total ERK1/2 Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

**Typical Data**

Representative results for the Phospho(Thr202/Tyr204; Thr185/Tyr187)/Total ERK1/2 Assay are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Western blot analyses of each lysate type were performed with phospho-ERK1/2 (Thr202/Tyr204; Thr185/Tyr187) and total ERK1/2 antibodies and are shown below for comparison.

Logarithmically growing Jurkat cells were treated with PMA (200 nM; 15 minutes) (positive) or LY294002 (50 μM; 2.5 hours) (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-ERK1/2 (Thr202/Tyr204; Thr185/Tyr187) antibody and anti-total ERK1/2 antibody on spatially distinct electrodes within a well. Phosphorylated and total ERK1/2 were detected with anti-total ERK1/2 antibody conjugated with MSD SULFO-TAG™ reagent.

![Graph showing Phospho/Total ERK1/2 + PMSF + SDS](image)

**Fig. 1:** Sample data generated with the MULTI-SPOT Phospho(Thr202/Tyr204; Thr185/Tyr187)/Total ERK1/2 Assay. Increased signal for phosphorylated ERK1/2 was observed with only pERK1/2 positive cell lysate. Total ERK1/2 signal increased throughout the titration of both pERK1/2 positive and negative cell lysates. The Phospho(Thr202/Tyr204; Thr185/Tyr187)/Total ERK1/2 Assay provides a quantitative measure of the data obtained with the traditional Western blot.
**Lysate Titration**

Data for pERK1/2 positive and negative Jurkat cell lysates using the MULTI-SPOT Phospho(Thr202/Tyr204; Thr185/Tyr187)/Total ERK1/2 Assay are presented below.

<table>
<thead>
<tr>
<th>Lysate (µg)</th>
<th>pERK1/2</th>
<th>ERK1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Average Signal</td>
<td>StdDev</td>
</tr>
<tr>
<td>0</td>
<td>142</td>
<td>11</td>
</tr>
<tr>
<td>0.078</td>
<td>1528</td>
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<tr>
<td>5.0</td>
<td>81470</td>
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</tr>
</tbody>
</table>

**MSD Advantage**

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample amounts of 25 µg/well 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 signal (light).
- **Simple protocols:** Only labels near the electrode surface are detected, enabling no-wash assays.
- **Flexibility:** Labels are stable, non-radioactive, and conveniently conjugated to biological molecules.
- **High sensitivity and precision:** Multiple excitation cycles of each label enhance light levels and improve sensitivity.

For a complete list of products, please visit our website at [www.mesoscale.com](http://www.mesoscale.com).

**References using MSD’s platform for the measurement of phosphoproteins**

