Phospho-Erk1/2 Pathway Sampler Kit

<table>
<thead>
<tr>
<th>Products Included</th>
<th>Product #</th>
<th>Quantity</th>
<th>Mol. Wt.</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospho-c-Raf (Ser338) (S646) Rabbit mAb</td>
<td>9427</td>
<td>20 µl</td>
<td>74 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Phospho-MEK1/2 (Ser217/221) (41G9) Rabbit mAb</td>
<td>9154</td>
<td>20 µl</td>
<td>45 kDa</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Phospho-p44/42 MAPK (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb</td>
<td>4370</td>
<td>20 µl</td>
<td>42, 44 kDa</td>
<td>Rabbit IgG</td>
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<tr>
<td>Phospho-MEK1/2 (Ser217/221) (41G9) Rabbit mAb</td>
<td>9154</td>
<td>20 µl</td>
<td>45 kDa</td>
<td>Rabbit IgG</td>
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<td>Phospho-c-Raf (Ser338) (S646) Rabbit mAb</td>
<td>9427</td>
<td>20 µl</td>
<td>74 kDa</td>
<td>Rabbit IgG</td>
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<tr>
<td>Phospho-p90RSK (Ser380) (D3H11) Rabbit mAb</td>
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<td>90 kDa</td>
<td>Rabbit IgG</td>
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<tr>
<td>Phospho-MEK1/2 (Ser217/221) (41G9) Rabbit mAb</td>
<td>9154</td>
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<td>Rabbit IgG</td>
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</tbody>
</table>

For Research Use Only. Not For Use In Diagnostic Procedures.

Description: The Phospho-Erk1/2 Pathway Sampler Kit provides an economical means of evaluating multiple members of the Erk pathway as well as their activation state. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs such as cell proliferation, differentiation, motility, and death. The p44/42 MAPK (ERK1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines (1-3) and is an important target in the diagnosis and treatment of cancer (4). Upon stimulation, a sequential three-part protein kinase cascade is initiated, consisting of a MAP kinase kinase (MAPKK), a MAP kinase kinase (MAPKKK), and a MAP kinase. While multiple ERK1/2 MAP3Ks have been identified, including the Raf family, Mos, and Tpl2/Cot, MEK1 and MEK2 are the primary MAPKKs in this pathway (5,6). MEK1 and MEK2 activate ERK1/p44 and ERK2/p42 through phosphorylation of activation loop residues Thr202/Tyr204 and Thr185/Tyr187, respectively. Several downstream targets of ERK1/2 have been identified, including p90RSK (7) and the transcription factor Elk-1 (8,9). ERK1/2 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases, known as DUSPs or MKPs (10), along with MEK inhibitors such as U0126 and PD98059.

Specificity/Sensitivity: Each antibody in the Phospho-Erk1/2 Pathway Sampler Kit recognizes only the phosphorylated form of its specific target.

Source/Purification: Polyclonal and monoclonal antibodies are produced by immunizing rabbits with synthetic phosphopeptides corresponding to residues surrounding Ser388 of human Raf, Ser217/221 of human MEK1/2, Thr202/Tyr204 of human p44 MAPK kinase, Ser380 of human p90RSK or Thr581 of human MSK1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Recommended Antibody Dilutions: Western blotting 1:1000

Please note Western blotting dilution for product #4370 is 1:2000.

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:
Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. 20X Phosphate Buffered Saline (PBS): (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH2O, mix.
2. 10X Tris Buffered Saline (TBS): (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH2O, mix.
3. 1X SDS Sample Buffer: Blue Loading Pack (#7722) or Red Loading Pack (#7723).
   Preparing fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH2O.
4. 10X Tris-Glycine SDS Running Buffer: (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH2O, mix.
5. 10X Tris-Glycine Transfer Buffer: (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH2O, mix.
6. 10X Tris Buffered Saline with Tween® 20 (TBST): (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH2O, mix.
7. Nonfat Dry Milk: (#9999)
8. Blocking Buffer: 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. Wash Buffer: (#9997) 1X TBST
10. Bovine Serum Albumin (BSA): (#9998)
11. Primary Antibody Dilution Buffer: 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
13. Prestained Protein Marker, Broad Range (Premixed Format): (#7720)
15. Secondary Antibody Conjugated to HRP: anti-rabbit (#7074); anti-mouse (#7076)
16. Detection Reagent: LumiGLO® chemiluminescent reagent and peroxide (#7720) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately aspirate the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). NOTES: Loading of prestained molecular weight markers (#7722), 10 µl/lane to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking
1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation
1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® 0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.