


# Anti-MEK1 + MEK2 (phospho S222) antibody ab4750

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## Overview

<b>Product name</b>	Anti-MEK1 + MEK2 (phospho S222) antibody
<b>Description</b>	Rabbit polyclonal to MEK1 + MEK2 (phospho S222)
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> ICC/IF, WB
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Human <b>Predicted to work with:</b> Chicken, Xenopus laevis, Chimpanzee 
<b>Immunogen</b>	Synthetic peptide corresponding to Human MEK1 + MEK2 (phospho S222).
<b>Positive control</b>	ICC/IF: Mouse embryonic fibroblasts WB: NIH3T3 cells
<b>General notes</b>	<p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&amp;As</p>

## Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
<b>Storage buffer</b>	Preservative: 0.05% Sodium azide Constituents: PBS, 0.1% BSA  BSA is IgG and protease free
<b>Purity</b>	Immunogen affinity purified
<b>Purification notes</b>	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated MEK 1 + 2. The final product is generated by affinity chromatography using a MEK 1 + 2 derived peptide that is phosphorylated at serine 222.

<b>Clonality</b>	Polyclonal
<b>Isotype</b>	IgG

## Applications

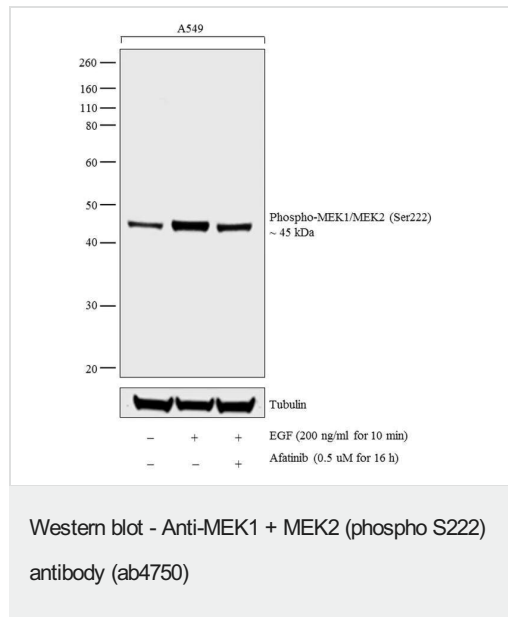
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab4750 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration. Assay dependent.
WB		1/1000. Predicted molecular weight: 43.3 kDa.

## Target

<b>Function</b>	Dual specificity protein kinase which acts as an essential component of the MAP kinase signal transduction pathway. Binding of extracellular ligands such as growth factors, cytokines and hormones to their cell-surface receptors activates RAS and this initiates RAF1 activation. RAF1 then further activates the dual-specificity protein kinases MAP2K1/MEK1 and MAP2K2/MEK2. Both MAP2K1/MEK1 and MAP2K2/MEK2 function specifically in the MAPK/ERK cascade, and catalyze the concomitant phosphorylation of a threonine and a tyrosine residue in a Thr-Glu-Tyr sequence located in the extracellular signal-regulated kinases MAPK3/ERK1 and MAPK1/ERK2, leading to their activation and further transduction of the signal within the MAPK/ERK cascade. Depending on the cellular context, this pathway mediates diverse biological functions such as cell growth, adhesion, survival and differentiation, predominantly through the regulation of transcription, metabolism and cytoskeletal rearrangements. One target of the MAPK/ERK cascade is peroxisome proliferator-activated receptor gamma (PPARG), a nuclear receptor that promotes differentiation and apoptosis. MAP2K1/MEK1 has been shown to export PPARG from the nucleus. The MAPK/ERK cascade is also involved in the regulation of endosomal dynamics, including lysosome processing and endosome cycling through the perinuclear recycling compartment (PNRC), as well as in the fragmentation of the Golgi apparatus during mitosis.
<b>Tissue specificity</b>	Widely expressed, with extremely low levels in brain.
<b>Involvement in disease</b>	Cardiofaciocutaneous syndrome 3
<b>Sequence similarities</b>	Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. MAP kinase kinase subfamily. Contains 1 protein kinase domain.
<b>Domain</b>	The proline-rich region localized between residues 270 and 307 is important for binding to RAF1 and activation of MAP2K1/MEK1.
<b>Post-translational modifications</b>	Phosphorylation at Ser-218 and Ser-222 by MAP kinase kinase kinases (RAF or MEKK1) positively regulates kinase activity. Also phosphorylated at Thr-292 by MAPK1/ERK2 and at Ser-298 by PAK. MAPK1/ERK2 phosphorylation of Thr-292 occurs in response to cellular adhesion and leads to inhibition of Ser-298 phosphorylation by PAK. Acetylation by Yersinia yopJ prevents phosphorylation and activation, thus blocking the MAPK signaling pathway.
<b>Cellular localization</b>	Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Cytoplasm, cytoskeleton, microtubule organizing center, spindle pole body. Cytoplasm. Nucleus. Localizes at centrosomes

## Images



**All lanes :** Anti-MEK1 + MEK2 (phospho S222) antibody (ab4750)

**Lane 1 :** A549 whole cell extract lysate

**Lane 2 :** A549 whole cell lysate treated with EGF (200 ng/mL for 10 min)

**Lane 3 :** A549 whole cell lysate treated with Afatinib (0.5 uM for 16 h) followed by EGF (200 ng/mL for 10 min)

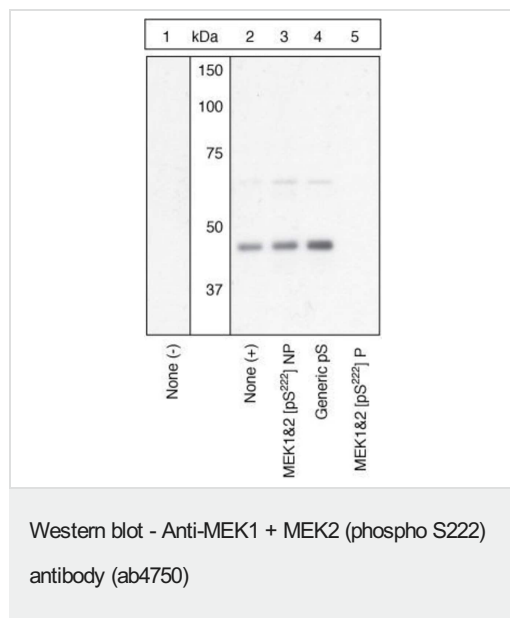
Lysates/proteins at 30 µg per lane.

### Secondary

**All lanes :** Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate at 0.25 µg/ml

**Predicted band size:** 43.3 kDa

Western blot analysis was performed on whole cell extracts of A549 in different conditions, observed a 45 kDa band corresponding to Phospho-MEK1/MEK2 (Ser222) was observed in untreated A549 lysate, the signal increased upon EGF treatment, and decreased upon pretreatment with the EGFR antagonist, Afatinib. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 4-12 % Bis-Tris gel, XCell SureLock™ Electrophoresis System and Novex® Sharp Pre-Stained Protein Standard. Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System. The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate.



**Lane 1 :** Anti-MEK1 + MEK2 (phospho S222) antibody (ab4750)

**Lane 1 :** Untreated NIH3T3 cells

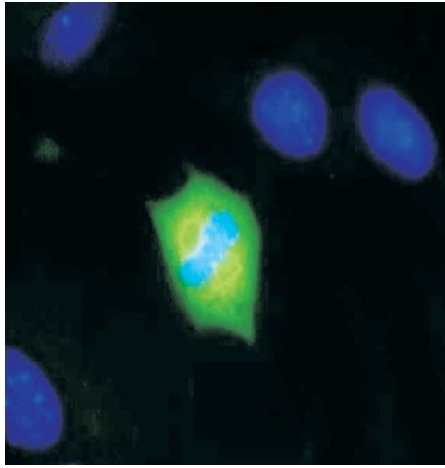
**Lanes 2-5 :** Anti-MEK1 + MEK2 (phospho S222) antibody (ab4750)

### Secondary

**Lane 1 :** A goat F (ab)2 anti-rabbit IgG alkaline phosphatase

**Predicted band size:** 43.3 kDa

Peptide Competition and Stimulation Extracts of NIH3T3 cells untreated (lane 1) or treated with 50 ng/mL PDGF for 15 minutes (2-5) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 4% BSA-TBST buffer overnight at 4°C, then incubated with the MEK1&2 (pS222) antibody for two hours at room temperature in a 1% BSA-TBST buffer, following various prior incubation conditions. After washing, the membrane was incubated with goat F (ab)2 anti-rabbit IgG alkaline phosphatase and signals were detected using the Pierce SuperSignal method. The data show that only the phosphopeptide corresponding to MEK1&2 (pS222) block the antibody signal, demonstrating the specificity of the antibody. The data also show the induction of MEK1&2 (pS222) phosphorylation by the addition of PDGF to this cell system.



Immunofluorescent analysis of mouse embryonic fibroblasts labelled for MEK1/2 (pS222) using ab4750, phosphospecific antibody. The cell is actively dividing. Blue represents chromosomes in anaphase of mitotic cell division. Green shows mitotic spindle expressing phosphorylated MEK1/2.

Immunocytochemistry/ Immunofluorescence - Anti-MEK1 + MEK2 (phospho S222) antibody (ab4750)

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