Phospho-4E-BP1 (Ser65) Antibody

Background: Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the eIF4E translation initiation factor. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt pathway and FRAP/mTOR kinase regulate 4E-BP1 activity (2,3). Multiple 4E-BP1 residues are phosphorylated in vivo (4). While phosphorylation by FRAP/mTOR on Thr37 and Thr46 does not prevent the binding of 4E-BP1 to eIF4E, it is thought to prime 4E-BP1 for subsequent phosphorylation at Ser65 and Thr70 (5).

Specificity/Sensitivity: Phospho-4E-BP1 (Ser65) Antibody detects endogenous levels of 4E-BP1 when phosphorylated at serine 65. This antibody has been shown to also recognize 4E-BP1 when phosphorylated at serine 101 (See application reference, Wang et al. 2003).

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to the sequence of mouse 4E-BP1. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.
**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:
Western blotting 1:1000
Immunoprecipitation 1:50

For application specific protocols please see the webpage for this product at www.cellsignal.com.

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

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Table:

<table>
<thead>
<tr>
<th>Applications</th>
<th>Species Cross-Reactivity*</th>
<th>Molecular Wt.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>W, IP</td>
<td>H, M, R, Mk</td>
<td>15-20 kDa</td>
<td>Rabbit**</td>
</tr>
</tbody>
</table>

Entrez-Gene ID # 1978
Swiss-Prot Acc. # Q13541

Western blot analysis of extracts from 293 cells using 4E-BP1 Antibody #9644 (lower) and Phospho-4E-BP1 (Ser65) Antibody #9451 (upper). The cells were starved for 24 hours in serum-free medium and underwent a 1 hour amino acid deprivation. Amino acids were replenished for 1 hour. Cells were then either untreated (-) or treated with 100 nM insulin (+) for 30 minutes.

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