Store at -20°C

#2845

4E-BP2 Antibody

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
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info@cellsignal.com
Web ■ www.cellsignal.com

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

Entrez-Gene ID #1979
Swiss-Prot Acc. #Q13542

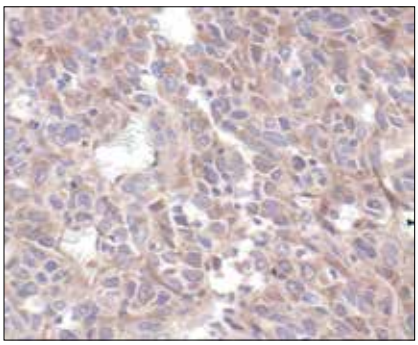
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IHC-P, F Endogenous	H, M, R, Mk, B	15 to 20 kDa	Rabbit**

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).

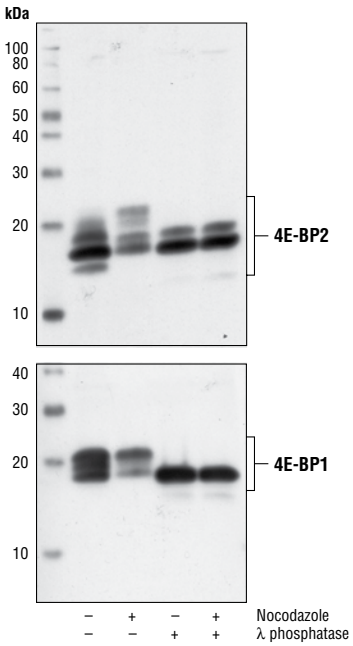
4E-BP2 and 4E-BP3 share high sequence homology with 4E-BP1, including conservation of the major FRAP/mTOR-dependent phosphorylation sites. Preliminary data suggests that phosphorylation of 4E-BP2 is regulated in a similar manner to that of 4E-BP1, although phosphorylation of this protein has not been as extensively studied (6).

Specificity/Sensitivity: 4E-BP2 Antibody detects endogenous levels of total 4E-BP2, independent of phosphorylation. This antibody does not cross-react significantly with 4E-BP1.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues at the carboxy-terminus of human 4E-BP2. Antibodies are purified by protein A and peptide affinity chromatography.



Immunohistochemical analysis of paraffin-embedded human follicular carcinoma (thyroid), using 4E-BP2 Antibody.



Western blot analysis of extracts from A673 cells, untreated or nocodazole-treated (100 ng/ml,16 hrs), using 4E-BP2 Antibody (upper) or 4E-BP1 Antibody #9452 (lower). Extracts were treated with λ phosphatase NEB#P0753 (10,000 U/ml for 1 hour) to dephosphorylate both proteins.

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.

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	1:100
Immunohistochemistry (Paraffin)	1:400
Unmasking buffer:	Citrate
Antibody diluent:	TBST-5%NGS
Flow Cytometry	1:200

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

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

Background References:

(1) Pause, A. et al. (1994) *Nature* 371, 762–767.

(2) Brunn, G.J. et al. (1997) *Science* 277, 99–101.

(3) Gingras, A.C. et al. (1998) *Genes Dev.* 12, 502–513.

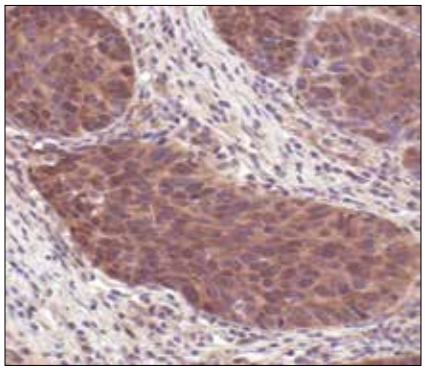
(4) Fadden, P. et al. (1997) *J. Biol. Chem.* 272, 10240–10247.

(5) Gingras, A.C. et al. (1999) *Genes Dev.* 13, 1422–1437.

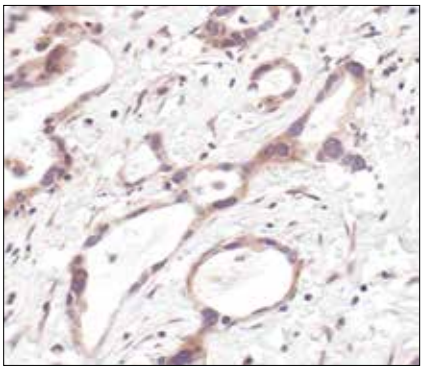
(6) Lin, T.A. and Lawrence, Jr, J.C. (1996) *J. Biol. Chem.* 271, 30199–30204.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

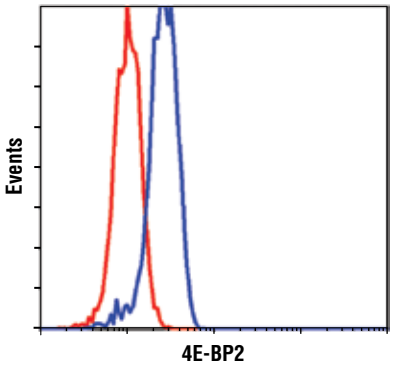
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



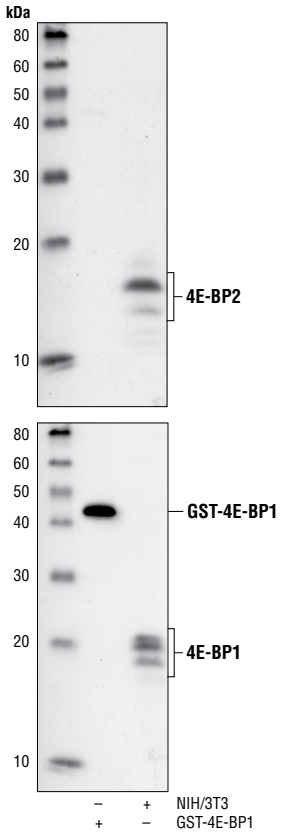
Immunohistochemical analysis of paraffin-embedded human colon carcinoma, showing cytoplasmic and nuclear localization, using 4E-BP2 Antibody.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma, using 4E-BP2 Antibody.



Flow cytometric analysis of HeLa cells, using 4E-BP2 Antibody (blue) compared to a nonspecifc negative control antibody (red).



Western blot analysis of bacterially expressed GST-4E-BP1 and of extracts from NIH/3T3 cells, using 4E-BP2 Antibody and 4E-BP1 Antibody #9452.