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4EBP1 Monoclonal Antibody (554R16)

Catalog Number **AHO1382**

Product data sheet

Details	
Size	100 µg
Host/Isotope	Mouse / IgG1, kappa
Class	Monoclonal
Type	Antibody
Clone	554R16
Immunogen	Recombinant human 4E-BP1 protein expressed in E. coli.
Conjugate	Unconjugated
Form	Liquid
Concentration	0.5 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.2, with 1% BSA
Contains	0.1% sodium azide
Storage Conditions	-20°C

Species Reactivity	
Species reactivity	Human, Mouse, Rat
Published species	Rat, Human, Not Applicable

Tested Applications	Dilution *
Western Blot (WB)	1:500
Immunocytochemistry (ICC/IF)	1:250

Published Applications	
Western Blot (WB)	See 2 publications below
Miscellaneous PubMed (Misc)	See 1 publications below
Immunocytochemistry (ICC/IF)	See 1 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Background/Target Information

This gene encodes one member of a family of translation repressor proteins. The protein directly interacts with eukaryotic translation initiation factor 4E, which is a limiting component of the multisubunit complex that recruits 40S ribosomal subunits to the 5' end of mRNAs. Interaction of this protein with eIF4E inhibits complex assembly and represses translation. This protein is phosphorylated in response to various signals including UV irradiation and insulin signaling, resulting in its dissociation from eIF4E and activation of mRNA translation.

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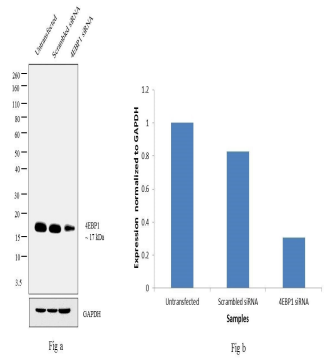
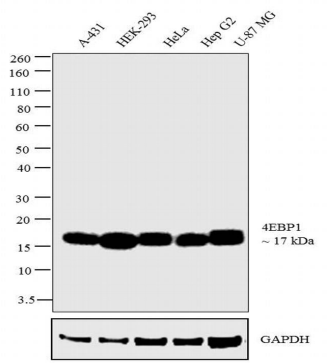
Product Images For 4EBP1 Monoclonal Antibody (554R16)



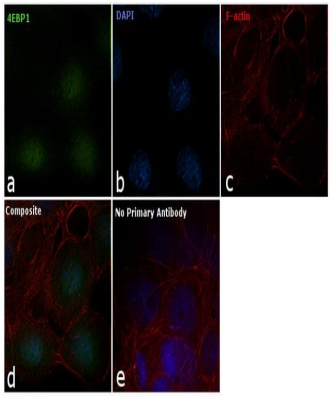
4EBP1 Antibody (AHO1382)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. A-431 cells were transfected with 4EBP1 siRNA and decrease in signal was observed in Western Blot using Anti-4EBP1 Monoclonal Antibody (Product # AHO1382). {KD}



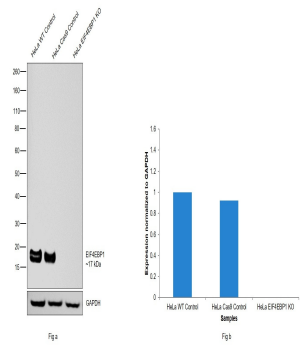
4EBP1 Antibody (AHO1382) in WB

Western blot analysis was performed on whole cell extracts of A-431 (Lane 1), HEK-293 (Lane 2), HeLa (Lane 3), Hep G2 (Lane 4) and U-87 MG (Lane 5). The blot was probed with Anti-4EBP1 antibody (Product # AHO1382, 1:500 dilution) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/mL, 1:4000 dilution). A 17 kDa band corresponding to 4EBP1 was observed across the cell lines tested.



4EBP1 Antibody (AHO1382) in ICC/IF

Immunofluorescence analysis of 4EBP1 was performed using 70% confluent log phase A-431 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with 4EBP1 Monoclonal Antibody (Product # AHO1382) at 1:250 dilution in 0.1% BSA and incubated overnight at 4 degree and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing nuclear and cytoplasmic localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



4EBP1 Antibody (AHO1382)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in EIF4EBP1 KO cell line compared to control cell line using Anti-4EBP1 Monoclonal Antibody (554R16)(Product # AHO1382). {KO}

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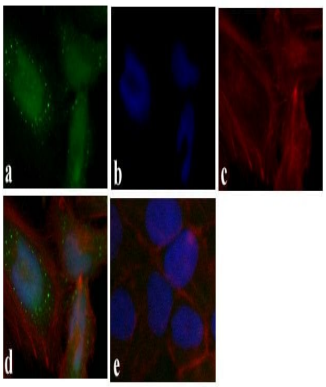
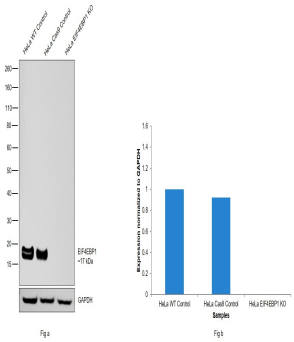


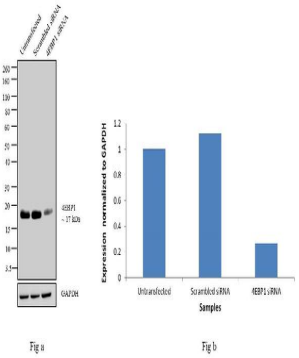
4EBP1 Antibody (AHO1382) in ICC/IF

Immunofluorescent analysis of 4E-BP1 was done on 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton™ X-100 for 10 minutes, and blocked with 5% BSA for 1 hour at room temperature. The cells were labeled with 4E-BP1 Mouse Monoclonal Antibody (Product # AHO1382) at 1:250 dilution in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor 488 Rabbit Anti-Mouse IgG Secondary Antibody (Product # A-11059) at a dilution of 1:400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor 594 Phalloidin (Product # A12381). Panel d is a merged image showing nuclear and perinuclear localization. Panel e shows no primary antibody control. The images were captured at 20X magnification.



4EBP1 Antibody (AHO1382) in WB

Knockout of EIF4EBP1 was achieved by CRISPR-Cas9 genome editing using LentiArray™ Lentiviral sgRNA (Product # A32042, AssayID CRISPR648136_LV) and LentiArray Cas9 Lentivirus (Product # A32064). Western blot analysis of EIF4EBP1 was performed by loading 30 µg of HeLa wild type (Lane 1), HeLa CAS9 (Lane 2) and HeLa EIF4EBP1 KO (Lane 3) whole cell extracts. The samples were electrophoresed using Novex® NuPAGE® 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Anti-4EBP1 Monoclonal Antibody (554R16)(Product # AHO1382) using 1:1,000 dilution and Goat anti-Mouse IgG (H+L), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A28177, 1:4,000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using SuperSignal™ West Dura Extended Duration Substrate (Product # 34076). Loss of signal upon CRISPR mediated knockout (KO) using the LentiArray™ CRISPR product line confirms that antibody is specific to EIF4EBP1.



4EBP1 Antibody (AHO1382) in WB

Knockdown of 4EBP1 was achieved by transfecting A-431 cells with 4EBP1 specific validated siRNAs (Silencer® select Product # s223471, s2234472). Western blot analysis (Fig. a) was performed using whole cell extracts from the 4EBP1 knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blots were probed with 4EBP1 Monoclonal Antibody (Product # AHO1382, 1:500 dilution) and Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/mL, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to 4EBP1.

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PubMed References For 4EBP1 Monoclonal Antibody (554R16)

2 Western Blot References

Species / Dilution	Summary
Human / 1:250	AHO1382 was used in Western Blotting to examine the effect of temozolomide (TMZ) combined with rapamycin on the TMZ-induced autophagic death of U251 glioma cells. Oncology letters (2018; 15: 2477) **"Effect and molecular mechanism of mTOR inhibitor rapamycin on temozolomide-induced autophagic death of U251 glioma cells."** Author(s):Li B,Zhou C,Yi L,Xu L,Xu M PubMed Article URL:http://dx.doi.org/10.3892/ol.2017.7537
Human / Not Cited	The Journal of biological chemistry (2010; 285: 39211) **"Butyrate-rich colonic microenvironment is a relevant selection factor for metabolically adapted tumor cells."** Author(s):Serpa J,Caiado F,Carvalho T,Torre C,Gonçalves LG,Casalou C,Lamosa P,Rodrigues M,Zhu Z,Lam EW,Dias S PubMed Article URL:http://dx.doi.org/10.1074/jbc.M110.156026

1 Miscellaneous PubMed References

Species / Dilution	Summary
Human / Not Cited	AHO1382 was used in western blot to report that cationic lipids induced autophagy in mammalian cells Autophagy (2010; 6: 449) **"Induction of genuine autophagy by cationic lipids in mammalian cells."** Author(s):Man N,Chen Y,Zheng F,Zhou W,Wen LP PubMed Article URL:http://dx.doi.org/10.4161/auto.6.4.11612

1 Immunocytochemistry References

Species / Dilution	Summary
Human / Not Cited	The Journal of biological chemistry (2010; 285: 39211) **"Butyrate-rich colonic microenvironment is a relevant selection factor for metabolically adapted tumor cells."** Author(s):Serpa J,Caiado F,Carvalho T,Torre C,Gonçalves LG,Casalou C,Lamosa P,Rodrigues M,Zhu Z,Lam EW,Dias S PubMed Article URL:http://dx.doi.org/10.1074/jbc.M110.156026

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