

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse 4EBP1.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human 4EBP1 Ser2-Ile118 Accession # Q13541
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Simple Western	5 µg/mL	See Below
Knockout Validated	4EBP1 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in 4EBP1 knockout HeLa cell line.	


DATA

Western Blot

Detection of Human/Mouse 4EBP1 by Western Blot.
Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and C2C12 mouse myoblast cell line. PVDF membrane was probed with 0.5 µg/mL of Human/Mouse 4EBP1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3227) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). Specific bands were detected for 4EBP1 at approximately 18-20 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Simple Western

Detection of Human 4EBP1 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for 4EBP1 at approximately 26 kDa (as indicated) using 5 µg/mL of Goat Anti-Human/Mouse 4EBP1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3227) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



Knockout Validated

Western Blot Shows Human 4EBP1 Specificity by Using Knockout Cell Line.
Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and 4EBP1 knockout HeLa cell line (KO). PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse 4EBP1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3227) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for 4EBP1 at approximately 17-20 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

New adjunct appears with knockout cell line.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

The eukaryotic initiation factor 4E (eIF-4E) binding protein 1 (4EBP1) binds to eIF-4E to prevent translation initiation. Upon phosphorylation by the target of rapamycin (TOR) kinase, 4EBP1 releases eIF-4E and translation initiation ensues. 4EBP1 is also known as PHAS-1 (phosphorylated heat and acid stable protein regulated by insulin 1).