Human SREBP2 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF7119

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human SREBP2 in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human (rh) SREBP1B and rhSREBP1C is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human SREBP2 Leu242-Asp450 Accession # Q12772
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.

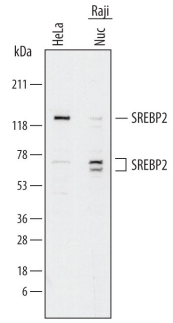
APPLICATIONS

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	2 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	Serum-starved HUVEC human umbilical vein endothelial cell chromatin, *ABCA1* promoter was detected by standard PCR	

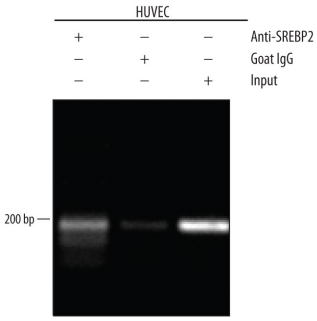
DATA

Western Blot



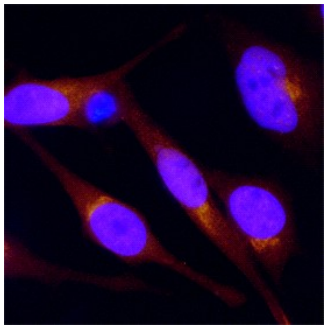
Detection of Human SREBP2 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and Raji human Burkitt's lymphoma cell line. Gels were loaded with 15 µg of nuclear extracts (Nuc). PVDF membrane was probed with 2 µg/mL of Goat Anti-Human SREBP2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7119) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for SREBP2 at approximately 125 kDa and 60-70 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Chromatin Immunoprecipitation (ChIP)



Detection of SREBP2-regulated Genes by Chromatin Immunoprecipitation. HUVEC human umbilical vein endothelial cells were serum-starved for 5 hours, fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. SREBP2/DNA complexes were immuno-precipitated using 5 µg Goat Anti-Human SREBP2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7119) or control antibody (Catalog # AB-108-C) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Goat IgG Secondary Antibody (Catalog # BAF109). Immuno-complexes were captured using 50 µL of MagCellect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *ABCA1* promoter was detected by standard PCR. In the figure, "Input" represents total cell lysate DNA.

Immunocytochemistry



SREBP2 in HeLa Human Cell Line. SREBP2 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Goat Anti-Human SREBP2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7119) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
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.** ● 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

SREBP2 (Sterol Regulatory Element-Binding Protein 2; also bHLHD2 and SREBF2) is a 120-125 kDa member of the SREBP family of proteins. It is ubiquitously expressed and found in the intracellular membrane fraction of cells. SREBP2 is a transcriptional factor initially embedded in the ER as an inactive precursor associated with SCAP. When necessary, SCAP mediates SREBP2 transfer to the Golgi, where two resident proteases remove the N-terminus from SREBP2, and the N-terminus is transported into the nucleus. Here, SREBP2 acts as a transcription factor, activating the LDLR and cholesterol synthesis genes. The human SREBP2 precursor is an 1141 amino acid (aa) two transmembrane protein whose N- and C-termini are cytoplasmic. The two cytoplasmic domains span aa 1-479 and 555-1141, respectively. Proteolytic cleavage between Leu484-Cys485 generates the 64-66 kDa SREBP2 transcription factor. This fragment contains a bHLH DNA binding domain (aa 330-380) and one Leu zipper region (aa 381-401). Homodimerization of SREBP2 is necessary for nuclear translocation. There is one potential isoform that shows a deletion of aa 274-276 coupled to a 96 aa substitution for aa 580-1141. A second isoform (known in rodent) shows a premature truncation after Val463 and runs at 55 kDa in SDS-PAGE. Over aa 242-450, human SREBP2 shares 97% aa identity with mouse SREBP2.

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