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Datasheet for 200-401-191

RFX5 Antibody

Overview

Description:	Anti-RFX5 (C-terminal) (RABBIT) Antibody - 200-401-191
Item No.:	200-401-191
Size:	100 µg
Applications:	ELISA, WB, ChIP, EMSA
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	DNA-binding protein RFX5 activates transcription from class II MHC promoters and recognizes X-boxes. It mediates cooperative binding between RFX and NF-Y. RFX binds the X1 box of MHC-II promoters. RFX5 may be associated with Bare Lymphocyte Syndrome Type Ii Complementation group C and Mhc Class Ii Deficiency. Anti-RFX5 Antibody is useful for researchers interested in Transcription factors, Tuberculosis, and Allograft rejection.
Synonyms:	rabbit anti-RFX5 antibody, DNA binding protein RFX 5 antibody, Influences HLA class II expression antibody, Regulatory factor X 5 antibody, Regulatory factor X subunit 5 antibody, Regulatory factor X 5 (influences HLA class II expression) antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	RFX5
Reactivity:	Human
Immunogen Type:	Conjugated Peptide
Immunogen:	RFX5 (C-terminal specific) peptide corresponding to a region near the C-terminus of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).

Purity/Specificity:	This product was prepared from monospecific antiserum by delipidation, salt fractionation and ion exchange chromatography. Anti-RFX5 (C-terminal specific) may react with a minor band at 68 kDa which may represent an RFX5 breakdown product or unmodified RFX5.
Relevant Links:	• NCBI - CAO72162.1 • UniProtKB - P48382 • GeneID - 5993

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	ChIP, EMSA (Based on references)
Application Note:	This product was assayed by immunoblot and found to be reactive against RFX5 (C-terminal specific) from a variety of fibroblast and B-cell lysates at a dilution of 1:250 to 1:500 followed by reaction with Peroxidase conjugated Affinity Purified anti-Rabbit IgG [H&L] (Goat) code #611-1302. Anti-RFX5 (C-terminal specific) is suitable for the detection by immunoblot of human RFX5 a shows a 75 kDa band. This product was also tested in a gel supershift assay and found to be reactive against RFX5 complexes using 0.5 to 1.0 µl per assay.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000 - 1:25,000
WB:	1:500 - 1:3,000

Formulation

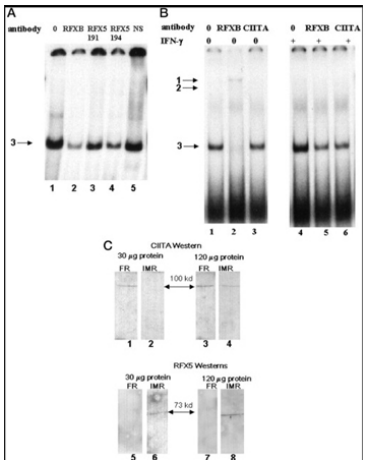
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

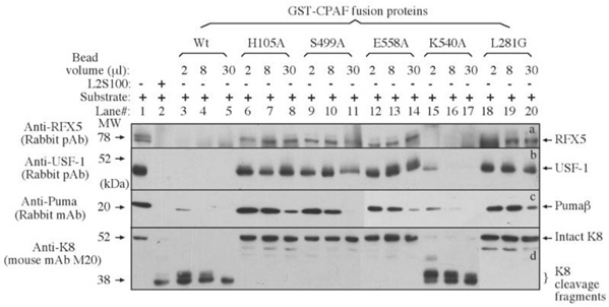
Shipping Condition:	Dry Ice

Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

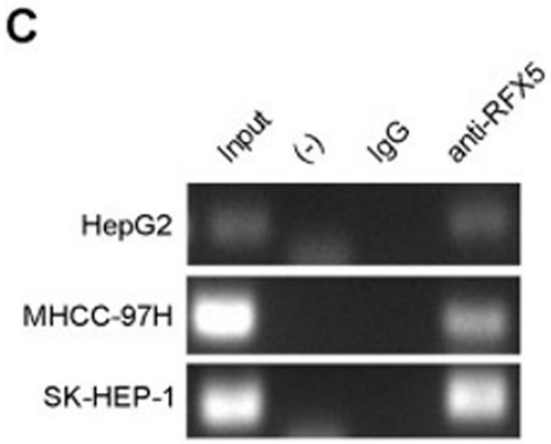


Western Blot
RFX5 complex at the collagen start site contains RFXB and RFX5 (A) and CIITA is present in the RFX5 complex generated with interferon-γ treated human fibroblast nuclear extract (B).
(A) specific antibodies to RFXB and RFX5 block complex 3 formation. Electrophoretic mobility shift assay was performed with unmethylated collagen probe in presence of a 50-fold excess of pB1 competitor and human fibroblast nuclear extract. Lane 1, no antibody; lane 2, RFXB antibody; lanes 3 and 4, RFX5 antibodies with different epitopes (200-401-191, 200-401-194); lane 5, non-immune IgG.
(B) electrophoretic mobility shift assay using unmethylated collagen probe and nuclear extracts from untreated (left panel, lanes 1–3) and treated (right panel, lanes 4–6) human fibroblasts (IMR-90). Control, lanes 1 and4; RFXB antibody, lanes 2 and 5; CIITA antibody, lanes 3 and 6. The arrows have the same meaning as in Fig. 1.
(C) RFX5 and low amounts of CIITA can be demonstrated in human fibroblast extracts whereas rat fibroblast extracts contain no RFX5 with higher amounts of CIITA. Western analysis of whole cell extracts from rat fibroblasts (FR, lanes 1, 3, 5, and 7) or human fibroblasts (IMR-90, lanes 2,4, 6, and 8) probed with CIITA antibody (top panels, lanes 1–4) or RFX5 antibody (p/n 200-401-194) (bottom panels, lanes 5–8). Western blots were performed using either 30 µg (lanes 1, 2,5, and 6) or 120 µg (lanes 3,4, 7, and 8) of total extracts.
Figure 4. PMID: 11986307



Western Blot

Degradation of target proteins by CPAF mutants in a cell-free assay. Either nuclear extract (NE) containing RFX5 (panel a) & USF-1 (b) or cytosolic extract (CE) containing Puma (c) and keratin 8 (d) were used as substrates to mix with the enzyme source (either L2S100 or various GST-CPAF fusion proteins) and the mixtures were incubated for 1 h at 37 °C. The entire mixture from each reaction was loaded into the corresponding lanes as indicated on top of the figure. After electrophoresis, the resolved protein bands were blotted onto nitrocellulose membrane for corresponding antibody detection as indicated along the left side of the figure. One independent set of enzyme/substrate reactions was used for detecting each substrate. The intact full-length keratin 8 has a molecular weight of 52 kDa and CPAF is known to cleave keratin 8 into 38 kDa fragments [17].
Figure 4. PMID: 19388144



Western Blot

RFX5 binds to the promoter region and activates transcription of the KDM4A genes in HCC cells.
(C) Immunoprecipitated DNA fragments produced by anti-RFX5 were subjected to PCR assay with primers that can detect the RFX5 binding peak site in the KDM4A promoter region which was determined by ChIP-seq. Consistent with ChIP-seq data, KDM4A promoter region was detected by PCR in the elution of anti-RFX5 in SK-HEP-1, MHCC-97H, and HepG2 cells, but not in the elution of control IgG (normal rabbit IgG p/n 011-0102). Figure 2. PMID: 32883983.

References

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- Chen D et al. Identifying catalytic residues in CPAF, a Chlamydia-secreted protease. *Arch Biochem Biophys.* (2009)
- Sengupta PK et al. The RFX Family Interacts at the Collagen (COL1A2) Start Site and Represses Transcription. *J Biol Chem.* (2002)
- Brickey WJ et al. Analysis of the defect in IFN-gamma induction of MHC class II genes in G1B cells: identification of a novel and functionally critical leucine-rich motif (62-LYLYLQL-68) in the regulatory factor X 5 transcription factor. *J Immunol.* (1999)
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