Datasheet for 200-401-194

RFX5 Antibody

Overview

Description:	Anti-RFX5 (aa 320 to 494) (RABBIT) Antibody - 200-401-194
Item No.:	200-401-194
Size:	100 µg
Applications:	WB, ChIP, EMSA, IF, IP
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Anti-RFX5 is specific for the RFX5 protein. RFX5 is the fifth member of the growing family of DNA-binding proteins sharing a novel and highly characteristic DNA-binding domain called the RFX motif. Multiple alternatively spliced transcript variants have been found but the full-length natures of only two have been determined. RFX5 has been shown to interact with CIITA.
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 peptide corresponding to a region at amino acids 320 to 494 of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).

Purity/Specificity:	RFXF5 antibody was prepared from monospecific antiserum by delipidation, salt fractionation and ion exchange chromatography. Anti-RFX5 (aa 320 to 494) may react with unknown minor bands at 65 kDa and 80 kDa.
Relevant Links:	• NCBI - CAO72162.1 • UniProtKB - P48382 • GeneID - 5993

Application Details

Tested Applications:	WB
Suggested Applications:	ChIP, EMSA, IF, IP (Based on references)
Application Note:	Anti RFX5 antibody has been assayed by immunoblot and found to be reactive against RFX5 (aa 320 to 494) from a variety of fibroblast and B-cell lysates at a dilution of 1:1,000 followed by reaction with Peroxidase conjugated Affinity Purified anti-Rabbit IgG. Anti-RFX5 (aa 320 to 494) detects a 75 kDa band by immunoblot for human RFX5. 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.
ChIP:	User Optimized
ELISA:	1:5,000 - 1:25,000
EMSA:	0.5 µL to 1.0 µL
WB:	1:1,000

Formulation

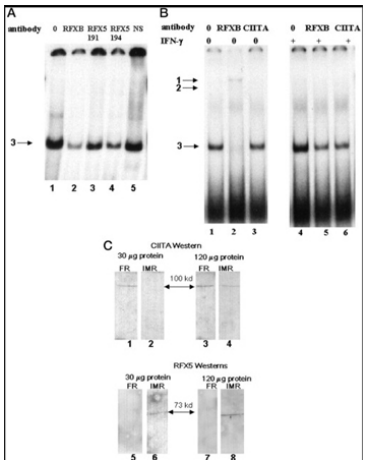
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

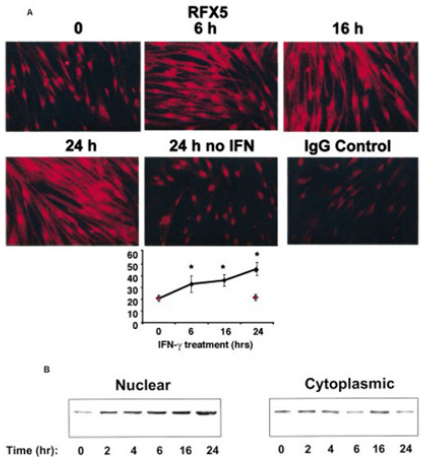
Shipping Condition:	Dry Ice

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.

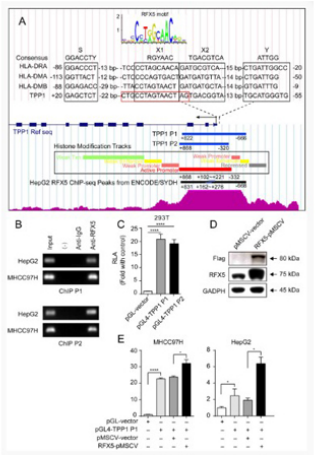
Images



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

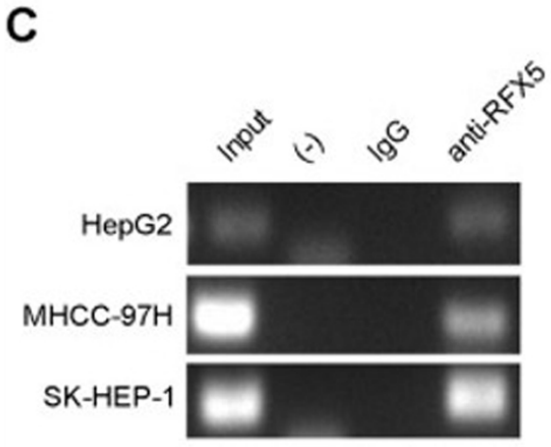


Immunofluorescence Microscopy
Protein levels of RFX5 increase in the nucleus during IFN-γ treatment.
A, RFX5 protein was detected using RFX5 (1:500) (194) primary antibody and Cy3-conjugated secondary antibody at 0, 6, 16, and 24 h after IFN-γ treatment or 24 h without IFN-γ (24h no IFN-γ). The last panel represents cells stained with IgG control (IgG). The mean intensity of fluorescence was analyzed using ImagePro Plus software. The black dots represent mean intensity of nuclei fluorescence in cells treated with IFN-γ and red dots represent mean intensity of nuclei fluorescence in cells treated without IFN-γ. The mean intensity of staining in the nucleus increased in a time-dependent manner. Three digitized images from three different experiments were analyzed by picking pixels that covered the nucleus. The program computed the average mean intensity in the nuclei and calculated the S.D. There was a significant increase (*, $p < 0.01$) in both RFX5 expression at all time periods (6, 16, 24 h) as indicated by analysis of variance (ANOVA) employing Scheffe's post-hoc procedures for RFX5 (graph at bottom of Fig. 6A).
B, a representative Western blot of proteins extracted from human lung fibroblasts treated with IFN-γ for different times (2, 4, 6, 16, and 24). Nuclear and cytoplasmic proteins (20 µg) were separated by 10% SDS gel electrophoresis, blotted, and detected by anti-RFX5 antibody. Fig. 6.
PMID: 12968017



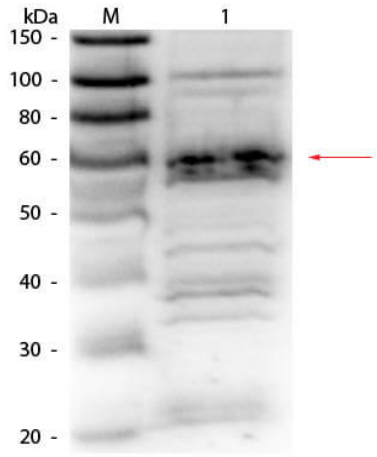
ChIP

RFX5 binds to the TPP1 promoter region and activates transcription of the TPP1 promoter. (A) The sketch of the TPP1 promoter region shows the RFX5 ChIP-seq binding peak in HepG2 cell and designed primers. Upper, RFX5 motif consensus sequence from ENCODE is shown, the S-Y motifs from HLA-DRA, HLA-DMA, HLA-DMB and TPP1 are boxed. Lower, (blue bold lines) the design of two PCR amplicons for the luciferase reporter gene, (Box) annotation of the TPP1 promoter based on the ENCODE Histone Modification Tracks, (light blue bold lines) the two ChIP-PCR amplicons and (purple peak) the RFX5 ChIP-seq binding peak in the TPP1 promoter. (B) ChIP-PCR assays showing RFX5 binding to the TPP1 promoter in HCC cells. Immunoprecipitated DNA fragments were analyzed by PCR using two independent primers mapped to the RFX5 ChIP-seq binding peak. (C) The transcriptional activity of two TPP1 promoter reporter constructs. Relative luciferase activity (RLA) was calculated as the ratio of firefly to Renilla luciferase activities to represent the promoter activity and performed in triplicate. The results represent one of three experiments. (D) Western blotting showing RFX5 protein with or without overexpression in 293T cells. (E) Luciferase assay to analyze the transcriptional impact of RFX5 on the TPP1 promoter in HCC cells. *P<0.05, ****P<0.0001.
Figure 2. PMID: 27840983

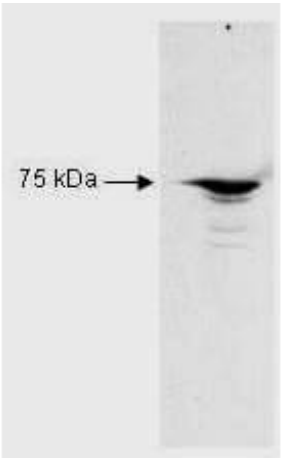


Western Blot

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.



Western Blot
Western Blot of Rabbit Anti-RFX5 antibody. Lane 1: Hela Nuclear Extract. Load: 25 µg per lane. Primary antibody: RFX5 antibody at 1:1,000 overnight at 4°C. Secondary antibody: HRP Goat-a-Rabbit IgG secondary antibody at 1:40,000 for 30 min at RT. Block: MB-070 for 30 min at RT. Predicted/Observed size: 65.3 kDa, 60 kDa for RFX5.



Western Blot
Western Blot of Rabbit Anti-RFX5 antibody. Lane 1: Raji B cell nuclear extract lysates. Lane 2: none. Load: 35 µg per lane. Primary antibody: RFX5 antibody at 1:2,500 for overnight at 4°C. Secondary antibody: HRP Goat-a-Rabbit IgG secondary antibody at 1:5,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 65.3 kDa, 72 kDa for RFX5. Other band(s): none.

References

- Nicosia L et al. Therapeutic targeting of EP300/CBP by bromodomain inhibition in hematologic malignancies. *Cancer Cell.* (2023)
- Chen, DB et al. RFX5 promotes the progression of hepatocellular carcinoma through transcriptional activation of KDM4A. *Scientific Reports* (2020)
- Wu X et al. Forkhead transcription factor FOXO3a mediates interferon-γ-induced MHC II transcription in macrophages. *Immunology* (2019)
- Zhao et al. The transcription factor RFX5 is a transcriptional activator of the TPP1 gene in hepatocellular carcinoma. *Oncology Reports* (2017)
- Dille et al. In contrast to Chlamydia trachomatis, Waddlia chondrophila grows in human cells without inhibiting apoptosis, fragmenting the Golgi apparatus, or diverting post-Golgi sphingomyelin transport. *Infection and Immunity* (2015)
- Xu Y et al. Interferon gamma repression of collagen (COL1A2) transcription is mediated by the RFX5 complex. *J Biol Chem.* (2003)
- Sengupta PK et al. The RFX Family Interacts at the Collagen (COL1A2) Start Site and Represses Transcription. *J Biol Chem.* (2002)

Disclaimer