

Datasheet for 100-401-249**CIITA Antibody****Overview**

Description:	Anti-CIITA aa 1-333 (RABBIT) Antibody - 100-401-249
Item No.:	100-401-249
Size:	100 µL
Applications:	WB, ChIP, IP
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Anti-CIITA antibody detects CIITA. The transactivator CIITA regulates basal and interferon-induced expression of Major Histocompatibility Complex class II genes. CIITA restores expression of all MHC class II gene expression in mutant cells and corrects regulatory defects of MHC class II genes. Antibodies to this transactivator are useful in the study of diseases of pathological MHC class II expression. Antigen can be obtained from Raji cell lysates. Typically levels of CIITA expression are too low to detect endogenous levels of protein expression. Transiently transfected cells are usually employed to study this transcription factor.
Synonyms:	rabbit anti-CIITA Antibody, MHC class II transactivator, CIITA, MHC2TA
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	CIITA
Reactivity:	Human
Immunogen Type:	Recombinant Protein
Immunogen:	The immunogen used for this study was a bacterially produced recombinant FLAG-CIITA corresponding to amino acids 1 through 333 of the human protein.

Purity/Specificity:	Anti-CIITA antibody was prepared from monospecific antiserum by delipidation and defibrination.
Relevant Links:	• UniProtKB - P33076 • NCBI - NP_000237.2 • GeneID - 4261

Application Details

Tested Applications:	WB
Suggested Applications:	ChIP, IP (Based on references)
Application Note:	Anti-CIITA antibody has been tested in western blot. For immunoblotting a 1:500 dilution is recommended. Researchers should determine optimal titers for other applications.
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

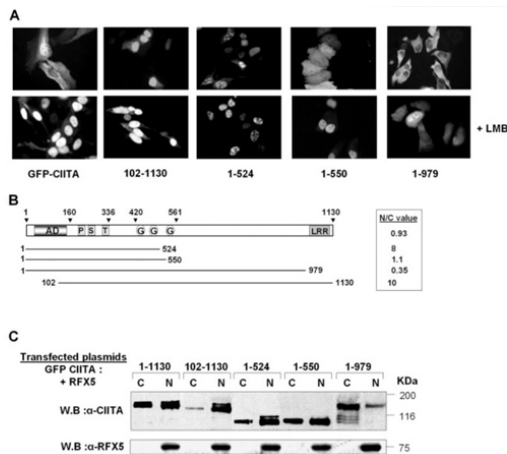
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	90 mg/mL by Refractometry
Buffer:	None
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.

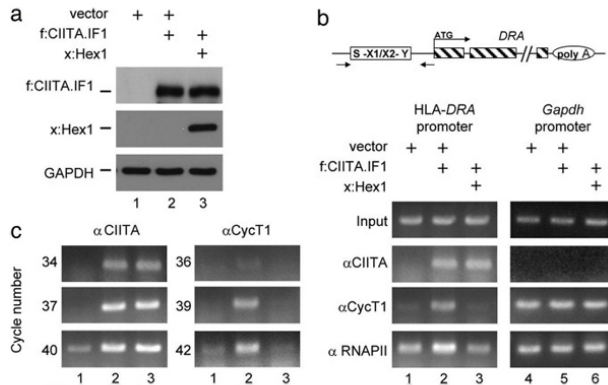
Images



Western Blot

Opposing effects of CIITA truncations on nuclear import and LMB mediated nuclear retention.

A. GFP fusions of the indicated regions of CIITA were transfected into HeLa cells. GFP-CIITA is the full-length (amino acids 1–1130) protein. Green fluorescence was observed 24 h later either without treatment or following a 2-h treatment with 20 nm leptomycin B (+LMB). B. schematic view of CIITA and truncation end points. The activation domain (AD), GTP binding motifs (GTP), nuclear import signals (NLS), and the leucine-rich region (LRR) are indicated. Shown on the right are the corresponding nuclear/cytoplasmic ratios obtained by the Scion Beta 4 image analysis program, and the values represent averages from at least 50 cells. C. cytoplasmic (C) and nuclear (N) fractions were prepared from HeLa cells transfected with 5 μ g of the indicated GFP CIITA derivatives and 1.5 μ g of an RFX5 expressing plasmid. Western blotting was performed using either α -CIITA (p/n 100-401-249) or α -RFX5 (p/n 100-401-193) antibodies. Fig 1. PMID: 11413136.

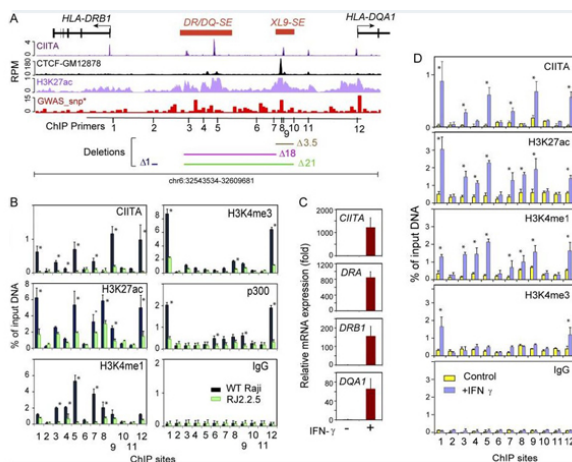


Immunoprecipitation

Hexim1 sequesters P-TEFb from CIITA on the endogenous HLA-DRA promoter.

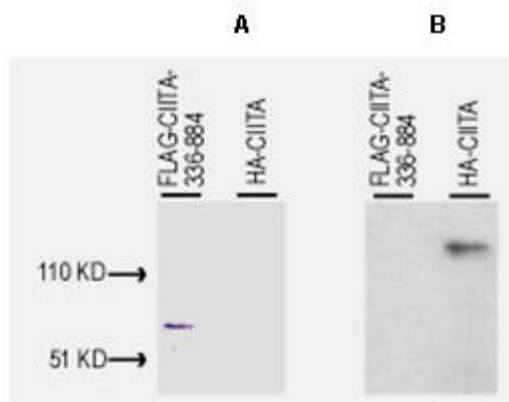
(a) Levels of f:CIITA,IF1 and x:Hex1 proteins in HeLa cells. f:CIITA,IF1 (1 μ g) was expressed alone or with x:Hex1 (1 μ g) in HeLa cells. The expression of these proteins was determined by Western blotting with α CIITA and α Xpress antibodies (Top and Middle). Levels of GAPDH were used to assess inputs of proteins for immunoblotting (Bottom). (b) ChIP assays were carried out with α CIITA, α CycT1, and α RNAPII antibodies. Sequences corresponding to the endogenous HLA-DRA promoter were detected by PCR. Additionally, the promoter for GAPDH gene was used as the negative control. Presented above the analyses is a diagrammatic representation of the HLA-DRA promoter and gene. Arrows below the diagram define positions of primers used in ChIP assays. Antibodies used in ChIP analyses are named next to the gels. (c) All results for PCR analyses were in the linear range of PCR. Amplifications with different numbers of cycles were carried out with α CycT1 and α CIITA immunoprecipitates. Numbers to the left depict actual number of cycles used in PCR.

Fig 4. PMID: 17088550



ChIP

Two SEs reside in the intergenic region between HLA-DRB1 and HLA-DQA1. (A) Schematic of the HLA-DR, -DQ intergenic region. CIITA, CTCF, and H3K27ac ChIP-seq data from The ENCODE Project Consortium (2012) and Scharer et al. (2015) are plotted with respect to the HLA-DRB1 and HLA-DQA1 genes. Red bars represent the DR/DQ-SE and XL9-SE SEs. The GWAS-SNP track shows the density of disease-associated SNPs in 500-bp bins across the region. The positions of ChIP primers (Table S1) are shown, as are regions that are deleted in the CRISPR/Cas9 mutant series. (B) Conventional ChIP-qPCR was performed on Raji and RJ2.2.5 cell chromatin for the indicated antibody with ChIP primer sets located at positions illustrated in A. (C) Relative fold change of the indicated gene expression as determined by qRT-PCR of A-431 cells $\pm$ 24 h treatment with IFN γ . (D) ChIP as in B of A-431 cells $\pm$ IFN γ treatment. Experiments in this figure were performed at least three times from independent cultures. Data represent mean $\pm$ SD, and significance was determined by Student's t tests. *, $P \leq 0.05$. Fig 1. PMID: 3175384.



Western Blot

Western blot of Anti-CIITA (1-333) antibody, generated by immunization with bacterially produced FLAG-CIITA aa 1-333, was tested by western blot against lysates of Cos-7 cells after transient transfection, separately, with pcDNA3-FLAG-CIITA-336-884 and pcDNA3-HA-CIITA. For transfection, Eugene 6 (Roche) was used according to the manufacturer's instructions for a 6-well plate format. Cells were lysed 24 h post-transfection in 200 μ L of 1x SDS-sample buffer, heated at 96°C for 5', and vortexed for 30 sec. Samples (10 μ L each) were separated on a 12% SDS-PAGE gel and transferred to PVDF (Millipore) followed by blocking for 45' using TTBS supplemented with 5% non-fat dry milk. All incubations were performed at room temperature. In panel A, both samples on PVDF were incubated with 10 μ g/mL mouse anti-FLAG antibody (Sigma) for 45'. After 5X washes with TTBS, reaction with ALP rabbit anti-mouse IgG at 200 ng/mL proceeded for 45' following again by washing as before. The blot was developed using BCIP/NBT. This blot demonstrates that FLAG-CIITA-336-884 was successfully over-expressed in the Cos-7 cells. In panel B, both samples on PVDF were incubated with a 1:500 dilution of Rockland's anti-CIITA (1-333) for 45'. After 5X washes with TTBS, reaction with HRP goat anti-rabbit IgG at 10 ng/mL proceeded for 45' following again by washing as before. The membrane was covered with Pico West Substrate solution (Pierce) for 5' and was then placed between the two layers of a standard sheet protector. Kodak O-MAT film was exposed to the blot for 30 sec and was immediately developed. The lane containing the lysate of pcDNA3-HA-CIITA transfected cells contains a single band at ~130 kDa molecular weight, whereas the lane containing lysate from pcDNA3-FLAG-CIITA-336-884 transfected cells shows no reactivity. This blot demonstrates that anti-CIITA (1-333) is specific for amino acids 1-333 of CIITA and that the antibody is not cross reactive with the FLAG portion of the immunogen.

References

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- Scharer et al. Genome-wide CIITA-binding profile identifies sequence preferences that dictate function versus recruitment. *Nucleic Acids Research* (2015)
- Uetani K et al. Influenza A virus abrogates IFN- γ response in respiratory epithelial cells by disruption of the Jak/Stat pathway. *Eur J Immunol.* (2008)
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