

Product Specification Sheet

Product: anti-CIITA aa 1-333 [Rabbit]

Code: 100-401-249

Lot # 11054

Size: 100 μ l

Physical State: Liquid (sterile filtered)

Protein Concentration: 90.0 mg/ml (by Refractometry)

Buffer: None

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

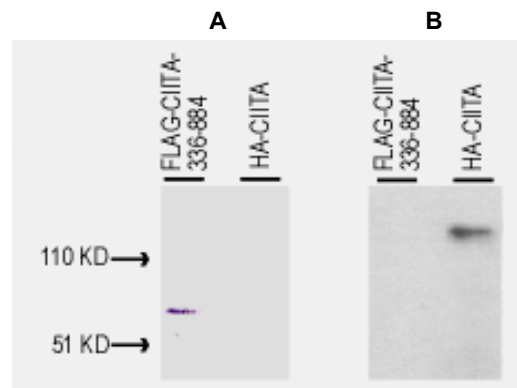
Application(s): Suitable for immunoblotting, ELISA and immunoprecipitation.

Figure 1. Immunoblot of anti-CIITA antibodies shows specificity.

Anti-CIITA (1-333) antibody, generated by immunization with bacterially produced FLAG-CIITA aa 1-333, was tested by immunoblot against lysates of Cos-7 cells after transient transfection, separately, with pcDNA3-FLAG-CIITA-336-884 and pcDNA3-HA-CIITA. For transfection, Fugene 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-336-842 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 kD molecular weight, whereas the lane containing lysate from pcDNA3-FLAG-CIITA-336-842 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



Background Information: 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.

Recommended Dilution(s): For immunoblotting a 1:500 dilution is recommended. Researchers should determine optimal titers for other applications.

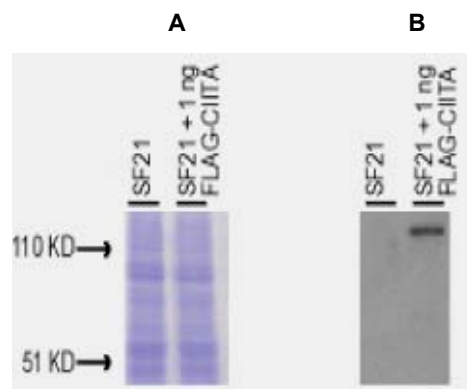
Storage Conditions: Store vial at -20° C or below prior to opening. Dilute only prior to immediate use. Aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is six (6) months from date of opening product.

Figure 2. Immunoblot of anti-CIITA antibodies shows sensitivity.

Anti-CIITA (1-333) antibody, generated by immunization with bacterially produced FLAG-CIITA aa 1-333, was tested by immunoblot against lysates from *Spodoptera frugiperda* 21 (SF21) cells (2×10^6 cells in 200 μ L of 1x SDS-sample buffer) and an SF21 lysate spiked with recombinant FLAG-CIITA to 0.2 ng/ μ L. Cells from both preparations were lysed by heating at 96°C for 5' and vortexing for 30 sec. Samples (5 μ L each) were separated on a 12% SDS-PAGE gel either stained with Coomassie Brilliant Blue or 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 of SF21 lysate and the SF21 lysate supplemented with CIITA are stained with Coomassie Brilliant Blue. The similar intensity of staining in each lane indicates that ~ equal amounts of total protein are present in both preparations. No band at the molecular weight of CIITA (130 KD) is distinguishable in the SF21 lysate supplemented with recombinant CIITA.

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 SF21 spiked with 1 ng of recombinant FLAG-CIITA (1-333) is clearly detectable as a single band at ~130 kD molecular weight, whereas no staining is evident in the control lysate of SF21 cells. This antibody may detect levels of CIITA below 1 ng when other detection techniques are used.



Purity: This product was prepared from monospecific antiserum by delipidation and defibrination.

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.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.