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Datasheet for 600-401-B04

Lac I Antibody

Overview

Description:	Anti-Lac I (RABBIT) Antibody - 600-401-B04
Item No.:	600-401-B04
Size:	100 µg
Applications:	ELISA, WB, IF, IP
Reactivity:	E. coli, Shigella sonnei
Host Species:	Rabbit

Product Details

Background:	Anti-Lac I antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. The lac repressor protein (Lac I) is an important genetic control protein. It controls the expression of the lactose metabolizing enzymes in Escherichia coli. This regulation involves specific recognition of the operator DNA sequence (Lac O) and consequent inhibition of transcription initiation by RNA polymerase; modulation of binding at this target site by small sugar molecules, inducers, provides the basis for regulation. Different ligand binding activities are associated with the isolated domains of Lac I: specific and nonspecific DNA binding with the NH2 termini, and inducer and specific DNA binding with the core protein. This antibody could possibly be used to recover and purify protein/nucleic acid complexes (host and viral proteins associated with the viral DNA) from infected cells.
Synonyms:	rabbit anti-LacI antibody, rabbit anti-Lac-I antibody, Rabbit anti-Lac I antibody, Lactose operon repressor, DNA binding transcriptional repressor antibody, Transcriptional repressor of the lac operon antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	LacI
Reactivity:	E. coli, Shigella sonnei

Immunogen Type:	Recombinant Protein
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a full-length recombinant Lac I protein.
Purity/Specificity:	This Anti-Lac I product was affinity purified from monospecific antiserum by immunoaffinity chromatography. This antibody reacts with recombinant Lac I protein. A BLAST analysis was used to suggest cross-reactivity with Lac I from DNA-binding transcriptional repressor in Escherichia coli (strain DH10B) and transcriptional repressor of the lac operon in Shigella sonnei (strain Ss046) based on a 100% homology with the immunizing sequence. Cross-reactivity with Lac I from other sources has not been determined.
Relevant Links:	• UniProtKB - P03023 • NCBI - 146576 • GeneID - 8156885

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IF, IP (Based on references)
Application Note:	Rabbit anti-Lac-I antibody has been tested for use in ELISA and western blotting using recombinant Lac I protein. Specific conditions for reactivity and detection of Lac I should be optimized by the end user. Expect a band approximately ~38 kDa in size corresponding to Lac I by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:90,000 – 1:100,000
WB:	1:500 - 1:2,000

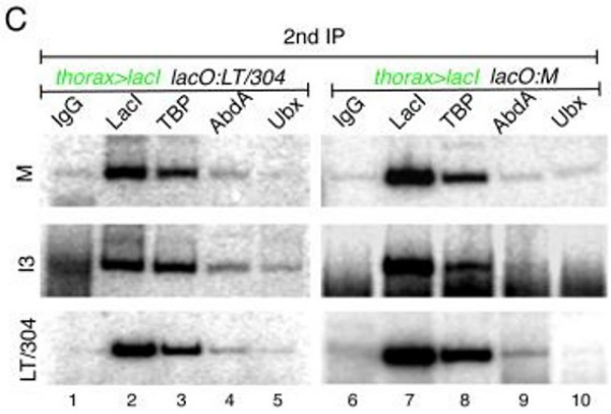
Formulation

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

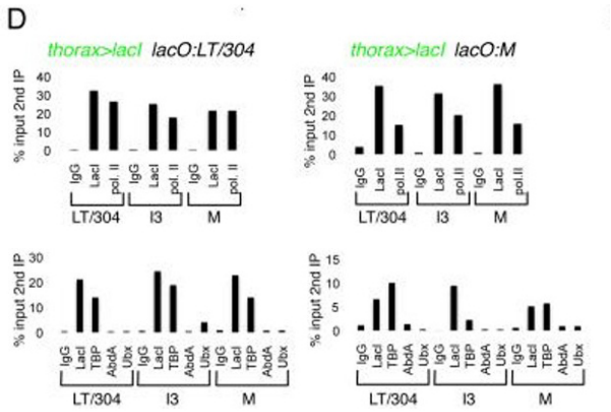
Shipping & Handling

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.

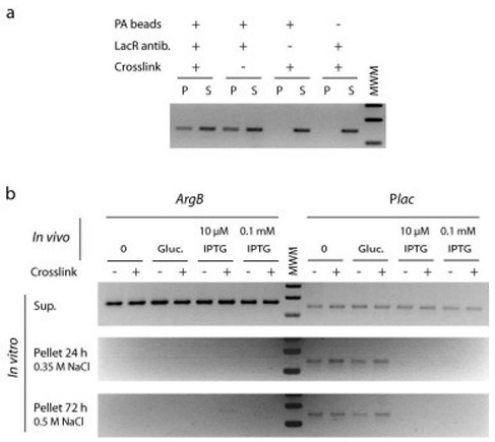
Images



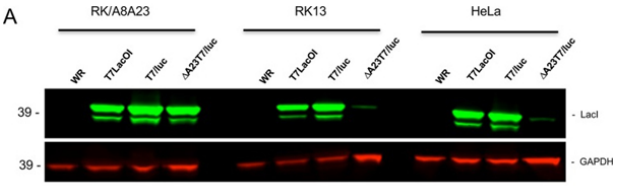
Immunoprecipitation
(C) 32P PCRs of cgChIPs from thorax>lacI; lacO:LT/304 (left) and thorax>lacI; lacO:M (right) embryos. These PCRs assess the presence of M, I3, and LT/304 sequences following a secondary IP using the antibodies indicated above the gels (IgG, anti-LacI, anti-TBP, anti-AbdA, and anti-Ubx). The results confirm that IPs for LacI and TBP, but not abdominal Hox proteins, pull-down multiple Dll 5' regions in Dll-expressing cells in the thorax. Fig 3. PMID: 22523743



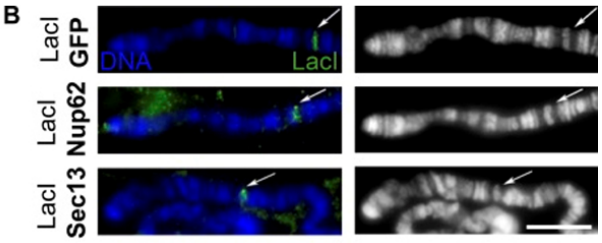
Immunoprecipitation
(D) qPCR measurements of cgChIP experiments for chromatin isolated from thorax>lacI; lacO:LT/304 (left) and thorax>lacI; lacO:M (right). Measurements are for the three Dll sequences (LT/304, I3, and M) after secondary IPs with the antibodies indicated (top gels: IgG, anti-LacI, anti-PolII; bottom gels: IgG, anti-LacI, anti-TBP, anti-AbdA, anti-Ubx). Quantifications are presented as percentages (%) of the qPCR signals obtained from PCRs for the same amplicons after the primary, anti-Flag IP (i.e.; % input 2nd IP). See also Supplementary Figure 1. Fig 3. PMID: 22523743



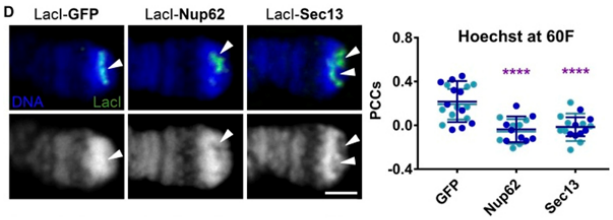
Immunoprecipitation
Stability of the LacI-LacO interaction. LacI-DNA complexes were prepared from E. coli K12 cells submitted or not to chemical crosslinking using formaldehyde, and the persistence of complexes was tested after two day-long washing. (a) Plac DNA fragment was immunoprecipitated by incubation of LacI antibody with an excess of cellular extracts (P, pellet; S, supernatant). Plac immunoprecipitation was no longer obtained in absence of protein A-coated beads (PA-beads), ruling out nonspecific adsorption to tube surfaces, and in absence of LacI antibody, ruling out nonspecific binding to the beads. (b) The persistence of LacI-Plac interaction was tested by high stringency washing of the DNA protein complexes attached to PA beads. A total of 4 tube transfers and 10 cycles of supernatant removal and replacement with fresh washing medium, were used for the final condition. Fig 2. PMID: 23454080



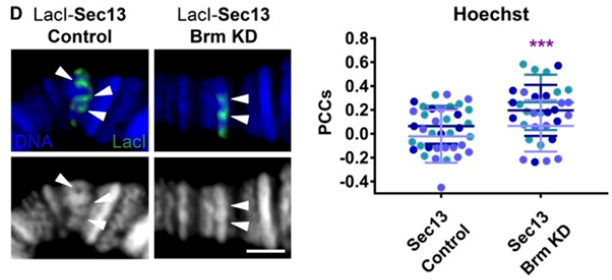
Western Blot
(A) RK/A8A23, RK13, and HeLa cells were infected with VACV WR (WR), T7LacOI, or T7/luc replication-competent virus or with replication-defective ΔA23T7/luc virus at a multiplicity of 5 PFU/cell. After 24 h, the cells were lysed and LacI was detected by SDS polyacrylamide gel electrophoresis followed by immunoblotting and probing with specific antibody. Antibody to GAPDH was used as a loading control. Fig 5. PMID: 28588133



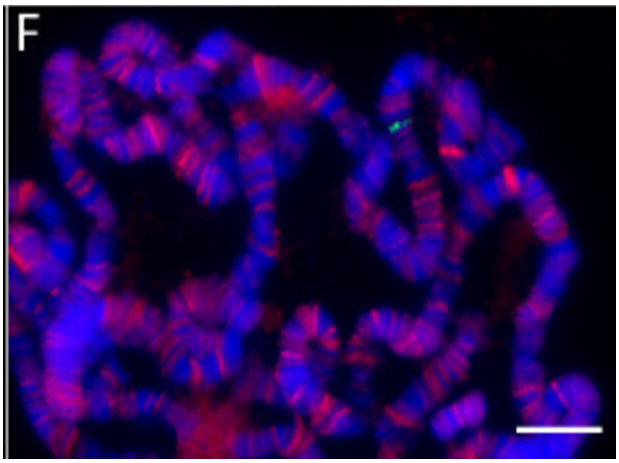
Immunofluorescence Microscopy
(B) Widefield IF of squashed polytene chromosomes with Hoechst stain (labeled DNA and shown as blue or white/gray here and hereafter) and α-LacI (green). The right column shows Hoechst only in grayscale, whereas the left column shows the overlay of both channels. Arrows point to the lacO integration site at location 4D5 near the end of the X chromosome. LacI-fusion protein expression was driven with second chromosome Nubbin-Gal4. The scale bar is 10 µm. Fig 1. PMID: 31366666



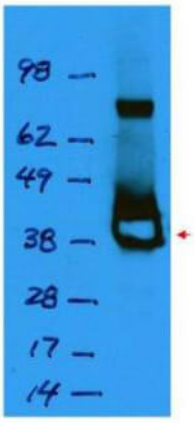
Immunofluorescence Microscopy
(D) Confocal IF images of LacI-fusion proteins targeted to the subtelomeric lacO integration site on squashed polytene chromosomes at location 60F stained with Hoechst (blue or white) and α-LacI (green). The top row shows the overlay of both channels, and the bottom row shows Hoechst only. "Holes" (areas of highly reduced staining density) in Hoechst staining can be reproducibly observed under LacI-Nup binding. Expression was driven with Sgs3-Gal4. Arrowheads show observed decondensation or lack thereof under LacI. The scale bar is 2 µm. Quantification displays PCCs between blue and green signal under LacI. Data are from two biological replicates (colored), each from an independent experiment. GFP, n = 19; Nup62, n = 15; Sec13, n = 17. ****, P < 0.0001. Error bars represent SDs. Fig 1. PMID: 31366666



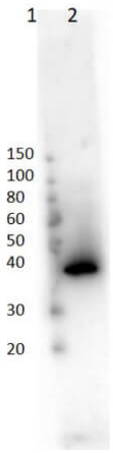
Immunofluorescence Microscopy
(D) Confocal IF images of LacI-Sec13 targeted to the lacO integration site on squashed polytene chromosomes at location 96C under control conditions (flies crossed to w1118 WT stock) or Brm KD conditions (flies crossed to Brm RNAi stock BL35211). Staining was with Hoechst (blue or white) and α-LacI (green). LacI-Sec13 protein expression and Brm RNAi were driven with Nubbin-Gal4. The top row shows the overlay of the two channels, whereas the bottom row shows DNA stain only in white/grayscale. Arrowheads indicate locations of observed decondensation or lack thereof under LacI signal. The scale bar is 2 µm. Quantification displays PCCs between red and blue signal under LacI. Data are from three biological replicates (colored) from two independent experiments. Sec13, n = 33. ***, P < 0.001. Error bars represent SDs. Fig 5. PMID: 31366666



Immunofluorescence Microscopy
F. Ideal squash and stain. Example includes lacO integration site at cytological site 96C, staining with antibodies against Nup98 (red, originally described in Capelson et al., 2010) and LacI (green, Rockland 600-401-B04), recognizing LacI-Sec13 fusion protein bound to lacO 96C, and Hoechst staining (blue). Scale bar = 10 µm. Fig 3. PMID: 33659343



Western Blot
Western blot using Rockland's affinity purified anti-Lac I antibody shows detection of a 38 kDa band corresponding to recombinant Lac I (arrowhead). The blot was blocked with 5% BLOTTO (p/n B501-0500) in TTBS overnight at 4°C. Primary antibody was used at a 1:1,000 dilution followed by reaction with a 1:10,000 dilution of HRP goat anti-rabbit IgG. ECL was used for detection. Personal communication, S. Hughes & M. Abram, NCI, Bethesda, MD.



Western Blot
Western Blot of Rabbit Anti-Lac I Antibody. Lane 1: Molecular Weight Marker (3µL). Lane 2: recombinant LAC-I (0.05µg). Primary Antibody: Anti-LAC-I at 1:1000 for 1hr at RT. Secondary Antibody: Goat anti-Rabbit IgG HRP (p/n 611-103-122) at 1:40,000 for 30mins at RT. Block: BlockOut Buffer (p/n MB-073) for 30mins at RT. Predicted/Observed MW: ~38kDa.

References

- Kuhn TM et al. Preparation of Drosophila Polytene Chromosomes, Followed by Immunofluorescence Analysis of Chromatin Structure by Multi-fluorescence Correlations. *Bio Proctoc.* (2020)
- Kuhn, TM et al. Chromatin targeting of nuclear pore proteins induces chromatin decondensation. *The Journal of Cell Biology* (2019)
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- Michel D et al. Kinetic approaches to lactose operon induction and bimodality. *J Theor Biol.* (2013)
- Agelopoulos M et al. Developmental regulation of chromatin conformation by Hox proteins in Drosophila. *Cell Rep.* (2012)

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