abcam

Product datasheet

Anti-DNA:RNA hybrid antibody [S9.6] ab234957

Recombinant

5 References   3 Images

Overview

Product name	Anti-DNA:RNA hybrid antibody [S9.6]
Description	Mouse monoclonal [S9.6] to DNA:RNA hybrid
Host species	Mouse
Specificity	The S9.6 monoclonal recognizes DNA-RNA hybrids (also known as R-loops) and does not bind to single or double stranded DNA (PubMed IDs: 2422282, 16614443). The antibody has high affinity for DNA-RNA hybrids but also binds RNA-RNA hybrids that are AU-rich (PubMed ID: 23784994). The specificity of the antibody appears to be determined by a combination of sequence and structural dependency since R-loop sequence affects binding affinity (PubMed ID: 28594954). It is important to use controls such as RNase A treatment in light of the cross-reactivity with double stranded RNA. Please see our DNA-RNA-IP (DRIP) protocol for further information. We do not recommend using this antibody for imaging applications such as ICC-IF, IHC or Flow due to the cross-reactivity with double stranded RNA.
Tested applications	**Suitable for:** IP, Dot blot **Unsuitable for:** ICC/IF or IHC-P
Species reactivity	**Reacts with:** Species independent
Immunogen	Chemical/ Small Molecule. This information is considered to be commercially sensitive.
Positive control	DRIP: R-Loop. Dot blot: R-Loop.
General notes	The pCALM3_2 vector used to generate R-loops was kindly provided by Prof. Frederic Chedin at UC Davis. The pCALM3_2 plasmid carries a 789bp fragment of the human CALM3 region that forms R-loops (PubMed ID: 31053798) cloned between T3 and T7 RNA polymerase promoter sequences. Transcription by T3 RNA polymerase leads to R-loop formation. Transcription by T7 RNA polymerase does not allow R-loop formation. Our DNA-RNA immunoprecipitation (DRIP) protocol is linked **here**. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	S9.6
Isotype	IgG2a
Light chain type	kappa

Applications

The Abpromise guarantee   Our **Abpromise guarantee** covers the use of ab234957 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		1/200. DNA-RNA hybrid Immunoprecipitation. Please find the DNA-RNA immunoprecipitation (DRIP) protocol link **here**.
Dot blot		1/1000.

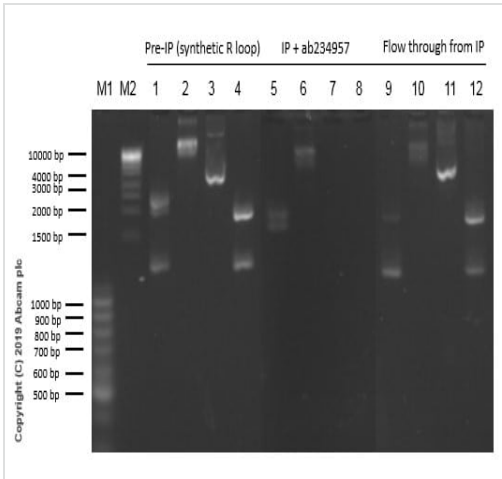**Application notes**   Is unsuitable for ICC/IF or IHC-P.

Target

Relevance   DNA-RNA hybrids occur naturally in eukaryotic cells, Particularly at sites of high transcriptional activity.

Images

Immunoprecipitation - Anti-DNA:RNA hybrid antibody [S9.6] (ab234957)

DNA-RNA hybrid Immunoprecipitation (DRIP) data using ab234957.

M1 : 100bp DNA ladder

M2 : High Range DNA Ladder

Lane 1: R-Loop ApaLI (RNase A treated)

Lane 2: R-Loop (RNase A treated)

Lane 3:R-Loop (RNase A+H treated)

Lane 4: R-Loop ApaLI (RNase A+H treated)

Lane 5: ab234957 with R-Loop ApaLI (RNase A treated)

Lane 6: ab234957 with R-Loop(RNase A treated)

Lane 7: ab234957 with R-Loop(RNase A+H treated)

Lane 8: ab234957 with R-Loop ApaLI (RNase A+H treated)

Lane 9-12: Flow through from IP

Capture antibody: 5 µg

Blocking buffer and concentration: 7.5 µg ssDNA.

Diluting buffer and concentration: PBS containing 0.1% Triton X-100.

A synthetic vector (pCALM3_2) was used to generate R-loops. ab234957 immunoprecipitates R-loops in the presence or absence of prior digestion by ApaLI, which does not affect R-loop structure. Prior treatment with RNase A (digests single stranded RNA) does not affect the IP signal whereas prior treatment with RNase H (digests RNA in DNA-RNA hybrids) eliminates the signal.

The pCALM3_2 vector used to generate R-loops was kindly provided by Prof. Frederic Chedin at UC Davis.
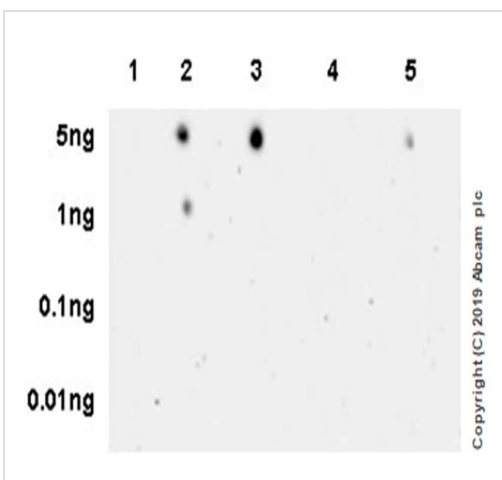
Dot Blot - Anti-DNA:RNA hybrid antibody [S9.6] (ab234957)

Dot blot analysis with ab234957 at 1/1000 dilution.

Lane 1: untranscribed plasmid.

Lane 2: R-Loop.

Lane 3: R-Loop (RNase A treated).

Lane 4: R-Loop (RNase A+H treated).

Lane 5: R-Loop (DNase I treated).

Goat Anti-Mouse IgG (H+L) at 1/10000 dilution was used as secondary antibody.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

pCALM3_2 was cloned in pFC53 in the T7 orientation, but it forms

R-loops transcribed in the reverse direction (that is with T3 promoter). DNase I has been shown to be less effective at digesting DNA in DNA-RNA hybrids compared to double stranded DNA (PubMed ID: 9020884). Prior treatment with DNase I reduced the signal by dot blot but did not eliminate it.

The pCALM3_2 vector used to generate R-loops was kindly provided by Prof. Frederic Chedin at UC Davis.

Why choose a recombinant antibody?

Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

Anti-DNA:RNA hybrid antibody [S9.6] (ab234957)

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