Overview

Product name
Anti-YY1 antibody [EPR4652] - Nuclear Loading Control and ChIP Grade

Description
Rabbit monoclonal [EPR4652] to YY1 - Nuclear Loading Control and ChIP Grade

Host species
Rabbit

Tested applications
Suitable for: Flow Cyt, WB, IP, IHC-P, ICC/IF, ChIP

Species reactivity
Reacts with: Mouse, Rat, Human

Immunogen
Synthetic peptide within Human YY1 aa 250-350 (internal sequence). The exact sequence is proprietary.

Positive control
Purchase matching WB positive control: Recombinant Human YY1 protein

WB: HeLa, Daudi, Y79, and HuT-78 cell lysates, mouse and rat heart tissue. IHC-P: Human kidney, tonsil and cervix carcinoma tissues. ICC/IF: HeLa and HUT-78 cells. IP: Y79 cells.

General notes
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

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.
Stable for 12 months at -20°C.

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol, 59% PBS, 0.05% BSA

Purity  
Protein A purified

Clonality  
Monoclonal

Clone number  
EPR4652

Isotype  
IgG

Applications

Our Abpromise guarantee covers the use of ab109237 in the following tested applications.

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

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<td>1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/250 - 1/500.</td>
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Target

Function  
Multifunctional transcription factor that exhibits positive and negative control on a large number of cellular and viral genes by binding to sites overlapping the transcription start site. May play an important role in development and differentiation. The function of YY1 as an activator or a repressor is specified by the presence of other proteins. For example it acts as a repressor in absence of adenovirus E1A protein but as an activator in its presence.

Sequence similarities  
Belongs to the YY transcription factor family. Contains 4 C2H2-type zinc fingers.

Cellular localization  
Nucleus matrix. Associated with the nuclear matrix.

Images
**Western blot** - Anti-YY1 antibody [EPR4652] - Nuclear Loading Control and ChIP Grade (ab109237)

All lanes: Anti-YY1 antibody [EPR4652] - Nuclear Loading Control and ChIP Grade (ab109237) at 1/10000 dilution (purified)

Lane 1: HeLa cell lysate
Lane 2: Daudi cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 45 kDa
Observed band size: 68 kDa

why is the actual band size different from the predicted?

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.

ab109237 staining YY1 in the human cell line HeLa (human cervix adenocarcinoma) by flow cytometry. Cells were fixed with 4% paraformaldehyde, permeabilised with 90% methanol and the sample was incubated with the primary antibody at a dilution of 1/30. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)
Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labelling YY1 with purified ab109237 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling YY1 with unpurified ab109237 at 1/100.
Chromatin was prepared from HT-29 cells according to the Abcam Dual X-ChIP protocol*. Cells were fixed with EGS for 30 minutes, then formaldehyde for 10 minutes.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab109237 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Primers and probes are located in the first kb of the transcribed region.

*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol

Western blot - Anti-YY1 antibody [EPR4652] - Nuclear Loading Control and ChIP Grade (ab109237)

All lanes: Anti-YY1 antibody [EPR4652] - Nuclear Loading Control and ChIP Grade (ab109237) at 1/50000 dilution (purified)

Lane 1: Y79 cell lysate
Lane 2: HuT-78 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 45 kDa
Observed band size: 68 kDa

why is the actual band size different from the predicted?

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis human tonsil tissue labelling YY1 with unpurified ab109237 at 1/250.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

All lanes: Anti-YY1 antibody [EPR4652] - Nuclear Loading Control and ChIP Grade (ab109237) at 1/2000 dilution (purified)

Lane 1: Mouse heart
Lane 2: Rat heart

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 45 kDa
Observed band size: 68 kDa why is the actual band size different from the predicted?

Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis human kidney tissue labelling YY1 with unpurified ab109237 at 1/250.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Immunocytochemistry/Immunofluorescence analysis of HUT-78 cells labelling YY1 with purified ab109237 at 1/50. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/50) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

All lanes: Anti-YY1 antibody [EPR4652] - Nuclear Loading Control and ChiP Grade (ab109237) at 1/1000 dilution (unpurified)

Lane 1: Daudi cell lysate
Lane 2: Y-79 cell lysate
Lane 3: HuT-78 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 45 kDa
ab109237 (purified) at 1/30 immunoprecipitating YY1 in Y79 cells. For western blotting, a Peroxidase-conjugated goat anti-rabbit IgG was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"