abcam

Product datasheet

Donkey Anti-Rabbit IgG H&L (Biotin) ab207999

6 References 4 Images

Overview

Product name Donkey Anti-Rabbit IgG H&L (Biotin)

Host species Donkey
Target species Rabbit

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

Immunogen The details of the immunogen for this antibody are not available.

Conjugation Biotin

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.

Avoid freeze / thaw cycle. Store In the Dark.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituents: PBS, 1% BSA, 30% Glycerol (glycerin, glycerine)

Purity Affinity purified

Purification notes Immunogen affinity purified - This antibody was isolated by affinity chromatography using antigen

coupled to agarose beads and conjugated to Biotin.

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab207999 in the following tested applications.

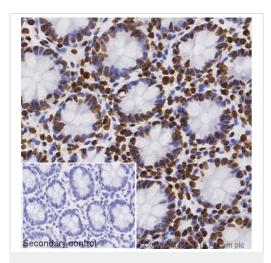
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ELISA		1/20000 - 1/200000.

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Application	Abreviews	Notes
IHC-Fr		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.
IHC-P		1/500 - 1/5000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Donkey Anti-Rabbit IgG H&L (Biotin) (ab207999)

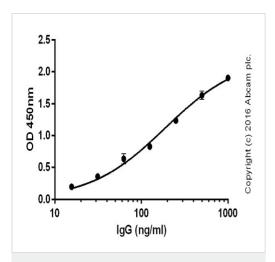
IHC image of Histone H4 staining in a section of formalin-fixed paraffin-embedded normal human colon tissue*. Ab207999 Donkey Anti-Rabbit IgG H & L (Biotin) was used as the secondary antibody.

Staining was performed on a Leica BondTM. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins, before blocking of endogenous biotin using **ab64212**. The section was then incubated with **ab177840**, 1/100 dilution, for 15 mins at room temperature, followed by ab207999, 1/2000 dilution, for 15 mins at room temperature. Detection was via an HRP conjugated ABC system and DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

The inset negative control image is taken from an identical assay without primary antibody.

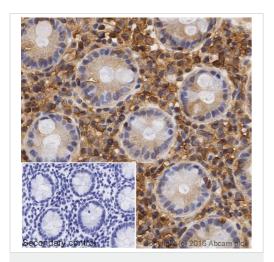
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



ELISA - Donkey Anti-Rabbit IgG H&L (Biotin) (ab207999)

ab207999 was tested by direct ELISA, where wells were coated with serially diluted rabbit IgG (1000 – 16 ng/ml) for 2 hours, followed by a 2 hour blocking step (5% BSA). ab207999 (1:20,000 dilution; 2 hours) was added and detected by streptavidin-HRP (ab7403; 1:10,000 dilution; 1 hour). Signal was developed by TMB substrate. Data from duplicates; +/- SD.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Donkey Anti-Rabbit IgG H&L (Biotin) (ab207999)

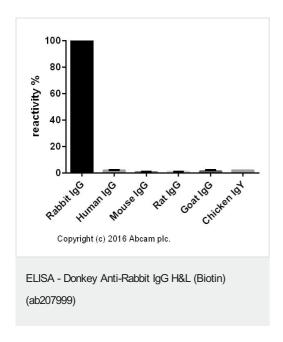
IHC image of beta Tubulin staining in a section of formalin-fixed paraffin-embedded normal human colon tissue*. Ab207999 Donkey Anti-Rabbit IgG H & L (Biotin) was used as the secondary antibody.

Staining was performed on a Leica BondTM. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins, before blocking of endogenous biotin using **ab64212**. The section was then incubated with **ab6046**, 1/100 dilution, for 15 mins at room temperature, followed by ab207999, 1/2000 dilution, for 15 mins at room temperature. Detection was via an HRP conjugated ABC system and DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Cross-reactivity of the polyclonal secondary antibody <u>ab182020</u> was tested using a sandwich ELISA approach. The wells were coated with the indicated IgG standards at 1 μ g/ml (50 μ l/well) and incubated overnight at 4°C, followed by a 5% BSA blocking step for 2h at RT. <u>ab182020</u> was then added starting at 1 μ g/ml and gradually diluted 1/4 (50 μ l/well), followed by incubation for 2h. For the detection Goat anti-Donkey IgG H&L (HRP) (<u>ab6988</u>) was used at 1/20,000 dilution (50 μ l/well), followed by incubation for 1h at RT.

For the batch tested, <u>ab182020</u> showed a cross-reactivity below 2% towards human IgG, mouse IgG, rat IgG, goat IgG and chicken IgY.

This data was developed using the unconjugated antibody (ab182020).

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