

## Product datasheet

# Donkey Anti-Rabbit IgG H&L (HRP) ab205722

★★★★★ [2 Abreviews](#) [19 References](#) [8 Images](#)

### Overview

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<b>Product name</b>	Donkey Anti-Rabbit IgG H&L (HRP)
<b>Host species</b>	Donkey
<b>Target species</b>	Rabbit
<b>Specificity</b>	The antibody used for conjugation reacts with rabbit immunoglobulins of all classes. Cross-reactions as determined by ELISA for the unconjugated antibody ( <a href="#">ab182020</a> ): Human IgG, mouse IgG, rat IgG, goat IgG and chicken IgY, less than 2%.
<b>Tested applications</b>	<b>Suitable for:</b> IHC-P, IP, WB, ELISA
<b>Immunogen</b>	The details of the immunogen for this antibody are not available.
<b>Conjugation</b>	HRP

### Properties

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<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	pH: 7.40 Preservative: 0.1% Proclin 300 Solution Constituents: PBS, 1% BSA, 30% Glycerol (glycerin, glycerine)
<b>Purity</b>	Immunogen affinity purified
<b>Purification notes</b>	This antibody was isolated by affinity chromatography using antigen coupled to agarose beads and conjugated to Horse Radish Peroxidase (HRP).
<b>Clonality</b>	Polyclonal
<b>Isotype</b>	IgG

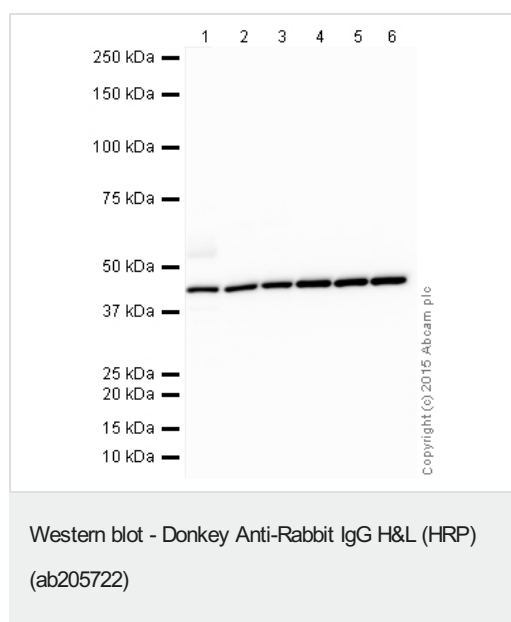
### Applications

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**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab205722 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
IHC-P		1/2000 - 1/20000.
IP		Use at an assay dependent concentration.
WB	★★★★★ (2)	1/2000 - 1/50000.
ELISA		Use at an assay dependent concentration.

## Images



**All lanes** : Anti-beta Actin antibody ([ab8227](#)) at 1 µg/ml

**Lane 1** : Liver (Human) Tissue Lysate

**Lane 2** : Liver (Mouse) Tissue Lysate

**Lane 3** : Liver (Rat) Tissue Lysate

**Lane 4** : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

**Lane 5** : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

**Lane 6** : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes** : Donkey Anti-Rabbit IgG H&L (HRP) (ab205722) at 1/10000 dilution

Developed using the ECL technique.

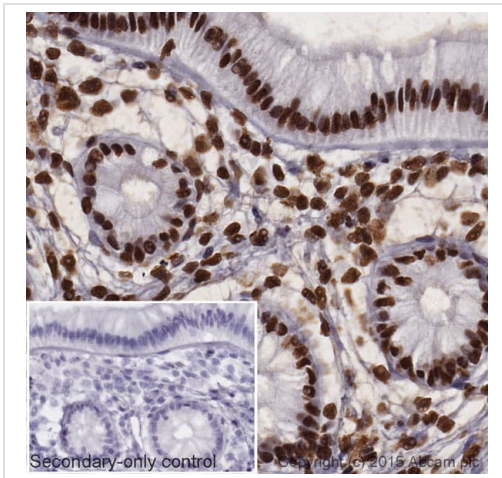
Performed under reducing conditions.

**Observed band size:** 42 kDa

**Exposure time:** 10 seconds

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine

Serum Albumin before being incubated with **ab8227** overnight at 4°C. Antibody binding was detected using ab205722, and visualised using ECL development solution **ab133406**.

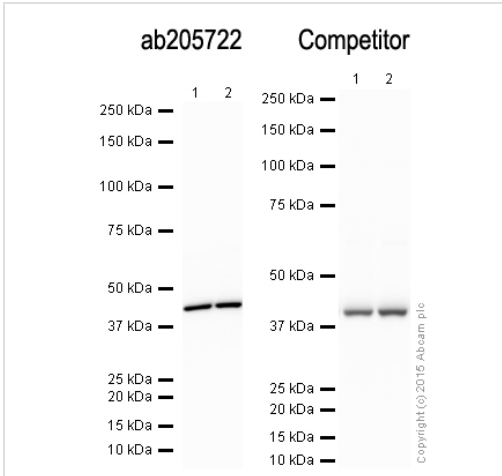


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Donkey Anti-Rabbit IgG H&L (HRP) (ab205722)

IHC image of Histone H4 staining in a section of formalin-fixed paraffin-embedded normal human colon tissue\*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins, and incubated overnight at +4°C with **ab177840** at 1ug/ml. An HRP-conjugated secondary (Ab205722, 1/10000 dilution) was used to detect the primary for 1hr at room temperature. DAB was used as the chromogen (**ab103723**), diluted 1/100 and incubated for 10min at room temperature. The section was 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



Western blot - Donkey Anti-Rabbit IgG H&L (HRP) (ab205722)

**All lanes** : Anti-beta Actin antibody (**ab8227**) at 1 µg/ml

**Lane 1** : Liver (Mouse) Tissue Lysate

**Lane 2** : Liver (Rat) Tissue Lysate

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes** : ab205722 (Left Image) at 1/10,000 and a competitor secondary (Right Image) at 1/10,000. Notice the decreased signal of the competitor product.

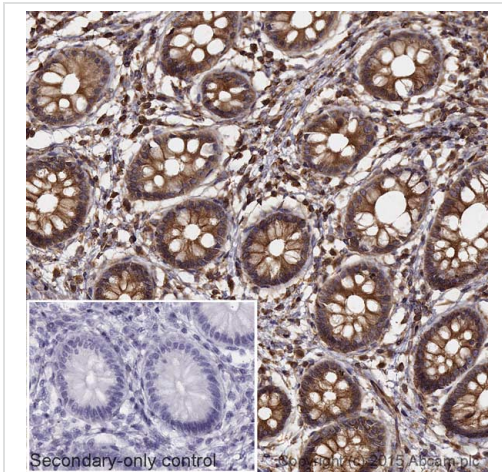
Performed under reducing conditions.

**Observed band size:** 42 kDa

**Exposure time:** 10 seconds

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being

transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with **ab8227** overnight at 4°C. Antibody binding was detected using ab205722 (Left Image) and a competitor secondary (Right Image), and visualised using ECL development solution **ab133406**.

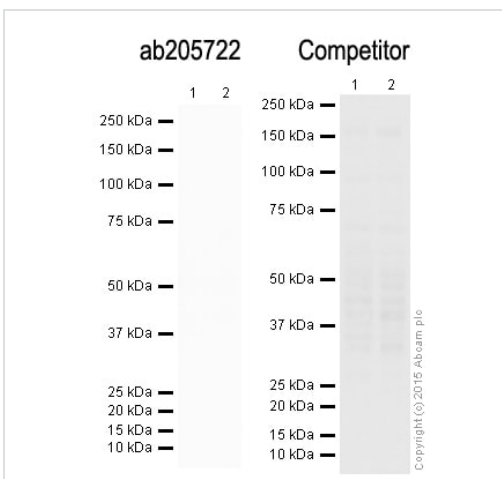


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Donkey Anti-Rabbit IgG H&L (HRP) (ab205722)

IHC image of beta tubulin staining in a section of formalin-fixed paraffin-embedded normal human colon tissue\*. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins, and incubated overnight at +4°C with **ab6046** at 5ug/ml. An HRP-conjugated secondary (Ab205722, 1/10000 dilution) was used to detect the primary for 1hr at room temperature. DAB was used as the chromogen (**ab103723**), diluted 1/100 and incubated for 10min at room temperature. The section was 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



Western blot - Donkey Anti-Rabbit IgG H&L (HRP) (ab205722)

**All lanes** : No Primary Antibody

**Lane 1** : Liver (Mouse) Tissue Lysate

**Lane 2** : Liver (Rat) Tissue Lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

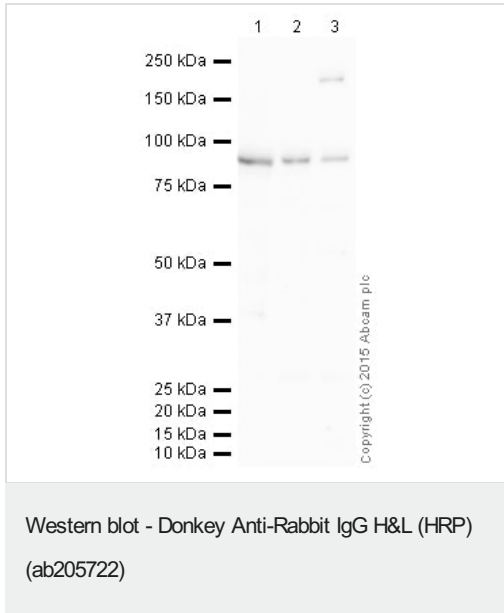
**All lanes** : ab205722 (Left Image) 1/2000 and a competitor secondary (Right Image) 1/2000. Notice the increased background of the competitor product.

Performed under reducing conditions.

**Exposure time:** 10 seconds

This blot was produced using a 4-12% Bis-tris gel under the MOPS

buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was incubated overnight with 2% Bovine Serum Albumin at 4°C. Any non-specific background binding was assessed by incubating the membrane with ab205722 (Left Image) and a competitor secondary (Right Image), and visualised using ECL development solution [ab133406](#).



**All lanes** : Anti-STAT3 antibody [EPR787Y] ([ab68153](#)) at 1/2000 dilution

**Lane 1** : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate

**Lane 2** : Heart (Mouse) Tissue Lysate

**Lane 3** : Heart (Rat) Tissue Lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

**All lanes** : Donkey Anti-Rabbit IgG H&L (HRP) (ab205722) at 1/2000 dilution

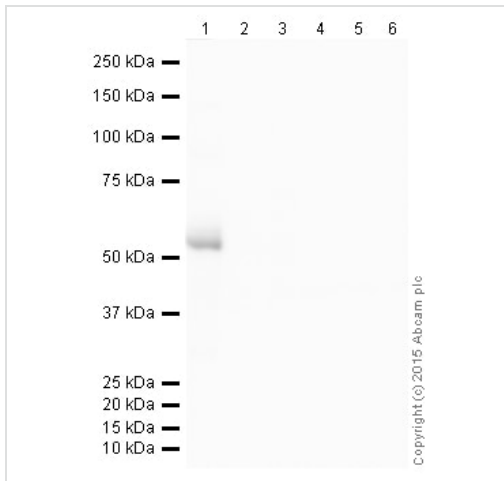
Developed using the ECL technique.

Performed under reducing conditions.

**Observed band size:** 88 kDa

**Exposure time:** 20 minutes

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with [ab68153](#) overnight at 4°C. Antibody binding was detected using ab205722, and visualised using ECL development solution [ab133406](#).



Western blot - Donkey Anti-Rabbit IgG H&L (HRP) (ab205722)

**All lanes** : No Primary Antibody

**Lane 1** : Liver (Human) Tissue Lysate

**Lane 2** : Liver (Mouse) Tissue Lysate

**Lane 3** : Liver (Rat) Tissue Lysate

**Lane 4** : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

**Lane 5** : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

**Lane 6** : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

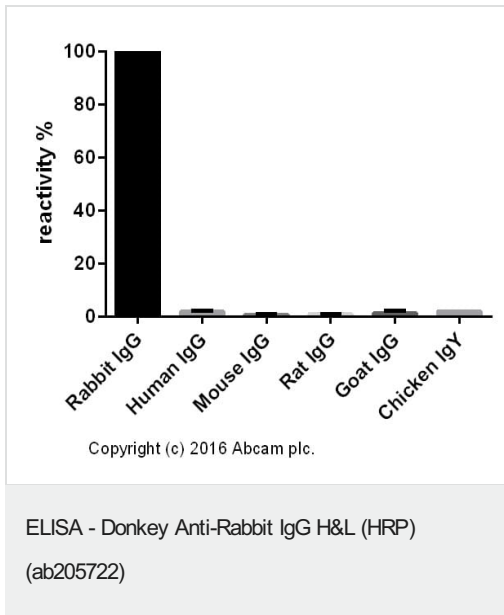
### Secondary

**All lanes** : Donkey Anti-Rabbit IgG H&L (HRP) (ab205722) at 1/2000 dilution

Performed under reducing conditions.

**Exposure time:** 10 seconds

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was incubated overnight with 2% Bovine Serum Albumin at 4°C. Any non-specific background binding was assessed by incubating the membrane with only the secondary antibody (ab205722), and visualised using ECL development solution [ab133406](#).



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

**For the batch tested, ab182020 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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