abcam

Product datasheet

Anti-HuD + HuC antibody [EPR19098] ab184267



★★★★★ 2 Abreviews 9 References 23 Images

Overview

Product name Anti-HuD + HuC antibody [EPR19098]

Description Rabbit monoclonal [EPR19098] to HuD + HuC

Host species Rahhit

Specificity This antibody shows very weak cross reactivity to HuB in WB test. Please contact our Scientific

support team for more information.

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

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse His-tagged HuC and His-GST-tagged HuD recombinant proteins; Human fetal brain;

> Mouse and rat brain; Neuro-2a and SH-SY5Y cell lysates. IHC-P: Human cerebral cortex, colon and glioma tissues; Mouse cerebral cortex and stomach tissues; Rat cerebral cortex and colon tissues. IHC-Fr: Mouse cerebral cortex and stomach tissues; Rat cerebral cortex and colon tissues. ICC/IF: SH-SY5Y and Neuro-2a cells. Flow Cyt: SH-SY5Y and Neuro-2a cells. IP: Mouse

brain lysate. I-ELISA: Mouse Elavl3 and 4.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

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

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

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

ClonalityMonoclonalClone numberEPR19098

Isotype IgG

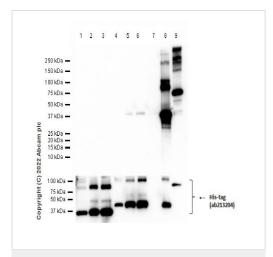
Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab184267 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	
Flow Cyt (Intra)		1/500.	
Indirect ELISA		Use a concentration of 1 µg/ml.	
WB		1/1000. Detects a band of approximately 39 kDa (predicted molecular weight: 40 kDa).	
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.	
IHC-Fr		1/500. Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20).	
ICC/IF		1/500.	
IP		1/30.	

Images		



Western blot - Anti-HuD + HuC antibody [EPR19098] (ab184267) **All lanes :** Anti-HuD + HuC antibody [EPR19098] (ab184267) at 1/1000 dilution

Lane 1 : His-tagged Mouse HUR recombinant protein (aa1-326) at 0.01 μg

Lane 2 : His-tagged Mouse HUR recombinant protein (aa1-326) at $0.05~\mu g$

Lane 3: His-tagged Mouse HUR recombinant protein (aa1-326) at 0.1 µg

Lane 4 : His-tagged mouse HuB recombinant protein (aa1-360) at 0.01 μg

Lane 5 : His-tagged mouse HuB recombinant protein (aa1-360) at 0.05 μg

Lane 6 : His-tagged mouse HuB recombinant protein (aa1-360) at 0.1 μg

Lane 7: Blank

Lane 8 : His-tagged mouse HuC recombinant protein (aa1-367) at 0.01 µg

Lane 9 : His-GST-tagged mouse HuD recombinant protein (aa1-385) at $0.01~\mu g$

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 40 kDa **Observed band size:** 37,75 kDa

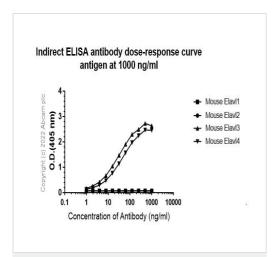
Exposure time: 10 seconds

Blocking and diluting buffer and concentration: 5%

NFDM/TBST.

ab184267 shows very weak cross reactivity with HuB.

Band around 80kda detected by anti-His tag antibody should be dimer.



Indirect ELISA - Anti-HuD + HuC antibody [EPR19098] (ab184267)

Indirect ELISA showing primary antibody ab184267 binding to Mouse Elavl1 (HuR); Mouse Elavl2 (HuB); Mouse Elavl3 (HuC); Mouse Elavl4 (HuD). Antigen concentration is 1000 ng/ml. Substrate solution is p-nitrophenyl phosphate(PNPP).

Binding of ab184267 was assessed in a serial dilution range 1000-0 ng/ml.

Binding was detected using the secondary antibody, Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit lgG(H+L) at 1/2500 dilution.



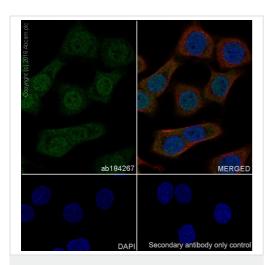
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HuD + HuC antibody
[EPR19098] (ab184267)

Immunohistochemical analysis of paraffin-embedded human cerebral cortex tissue labeling HuC + HuD with ab184267 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and weakly cytoplasmic staining on neurons of human cerebral cortex is observed [PMID: 22007133].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-HuD + HuC antibody [EPR19098] (ab184267)

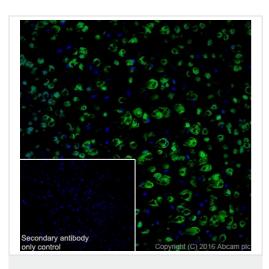
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SH-SY5Y (Human neuroblastoma cell line from bone marrow) cells labeling HuC + HuD with ab184267 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic and nuclear staining on SH-SY5Y cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab195889</u> (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.



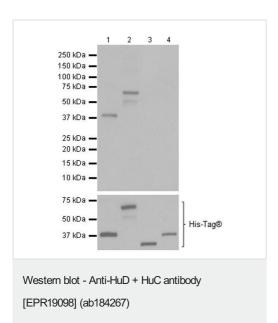
Immunohistochemistry (Frozen sections) - Anti-HuD + HuC antibody [EPR19098] (ab184267)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse cerebral cortex tissue labeling HuC + HuD with ab184267 at 1/500 dilution, followed by Goat anti-rabbit $\lg G$ (Alexa Fluor 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Cytoplasmic and nuclear staining on neurons of mouse cerebral cortex is observed.

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



All lanes : Anti-HuD + HuC antibody [EPR19098] (ab184267) at 1/2000 dilution

Lane 1: His-tagged mouse HuC recombinant protein (aa1-367)Lane 2: His-GST-tagged mouse HuD recombinant protein (aa1-385)

Lane 3: His-tagged mouse HuR recombinant protein (aa1-360)
Lane 4: His-tagged mouse HuB recombinant protein (aa1-360)

Lysates/proteins at 0.01 µg per lane.

Secondary

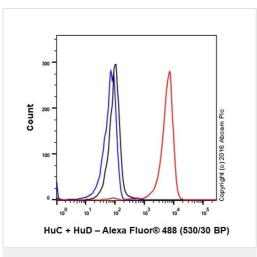
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 40 kDa Observed band size: 39,68 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

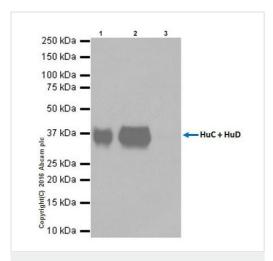
Exposure time: 0.5 second.

Please note that the band around 55kDa in lane 2 is most likely the \mbox{HuD} protein with tags.



Flow Cytometry (Intracellular) - Anti-HuD + HuC antibody [EPR19098] (ab184267)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed SH-SY5Y (Human neuroblastoma cell line from bone marrow) cells labeling HuC + HuDwith ab184267 at 1/500 dilution (red) compared with a rabbit monoclonal lgG isotype control (ab172730; black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit lgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-HuD + HuC antibody [EPR19098] (ab184267)

HuC + HuD was immunoprecipitated from 0.35 mg of mouse brain lysate with ab184267 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab184267 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

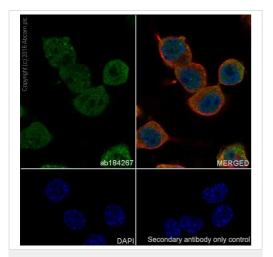
Lane 1: Mouse brain lysate, 10 µg (Input).

Lane 2: ab184267 IP in mouse brain lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab184267 in mouse brain lysate.

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

Exposure time: 30 seconds.



Immunocytochemistry/ Immunofluorescence - Anti-HuD + HuC antibody [EPR19098] (ab184267)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Neuro-2a (Mouse neuroblastoma cell line) cells labeling HuC + HuD with ab184267 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

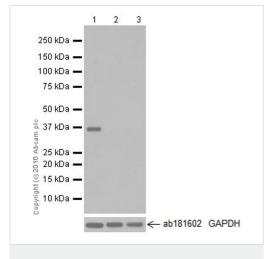
Confocal image showing cytoplasmic and nuclear staining on Neuro-2a cell line.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab195889</u> (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor®

488) (ab150077) at 1/1000 dilution.



Western blot - Anti-HuD + HuC antibody [EPR19098] (ab184267)

All lanes : Anti-HuD + HuC antibody [EPR19098] (ab184267) at 1/1000 dilution

Lane 1 : Human fetal brain lysate

Lane 2 : Human fetal heart lysate

Lane 3 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

Secondary

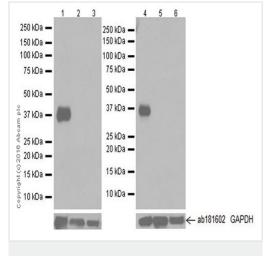
All lanes : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/4000 dilution

Predicted band size: 40 kDa **Observed band size:** 39 kDa

Exposure time: 8 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile is consistent with what has been described in the literature (PMID: 9016658; PMID:8535975).



Western blot - Anti-HuD + HuC antibody [EPR19098] (ab184267)

All lanes : Anti-HuD + HuC antibody [EPR19098] (ab184267) at 1/1000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Mouse heart lysate
Lane 3 : Mouse kidney lysate
Lane 4 : Rat brain lysate

Lane 5 : Rat heart lysate

Lane 6 : Rat kidney lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 40 kDa **Observed band size:** 39 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-3: 1 minute; Lane 4-6: 5 seconds.

The expression profile is consistent with what has been described in the literature (PMID: 9016658; PMID: 8535975).

All lanes : Anti-HuD + HuC antibody [EPR19098] (ab184267) at 1/5000 dilution

Lane 1 : Neuro-2a (Mouse neuroblastoma cell line) whole cell lysate

Lane 2: SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate

Lysates/proteins at 20 µg per lane.

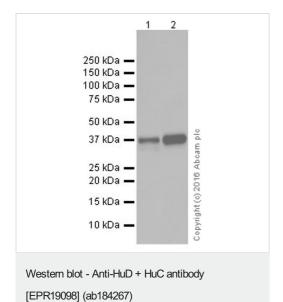
Secondary

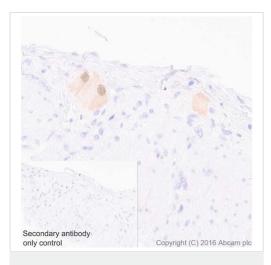
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 40 kDa **Observed band size:** 39 kDa

Exposure time: 5 seconds

Blocking/Dilution Buffer: 5% NFDM/TBST





Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HuD + HuC antibody
[EPR19098] (ab184267)

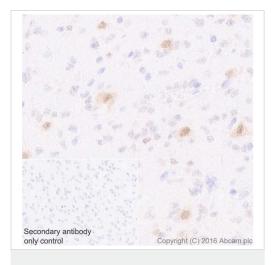
Immunohistochemical analysis of paraffin-embedded human colon tissue labeling HuC + HuD with ab184267 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and weakly cytoplasmic staining on myenteric ganglia of human colon is observed [PMID: 16918730].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



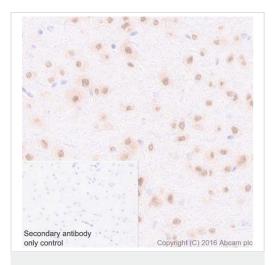
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HuD + HuC antibody
[EPR19098] (ab184267)

Immunohistochemical analysis of paraffin-embedded human glioma tissue labeling HuC + HuD with ab184267 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and weakly cytoplasmic staining on human glioma is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HuD + HuC antibody
[EPR19098] (ab184267)

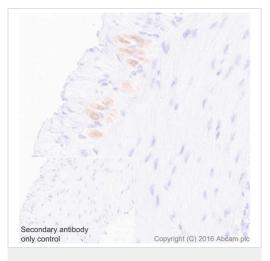
Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue labeling HuC + HuD with ab184267 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and weakly cytoplasmic staining on neurons of mouse cerebral cortex is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



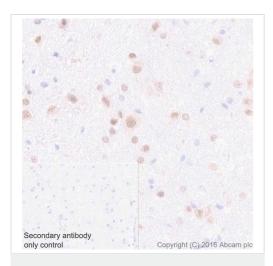
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HuD + HuC antibody
[EPR19098] (ab184267)

Immunohistochemical analysis of paraffin-embedded mouse stomach tissue labeling HuC + HuD with ab184267 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic and nuclear staining on myenteric ganglia of mouse stomach is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HuD + HuC antibody
[EPR19098] (ab184267)

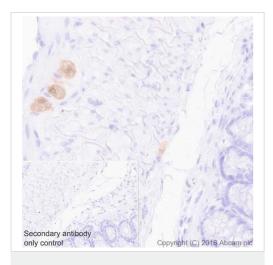
Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue labeling HuC + HuD with ab184267 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and weakly cytoplasmic staining on neurons of rat cerebral cortex is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



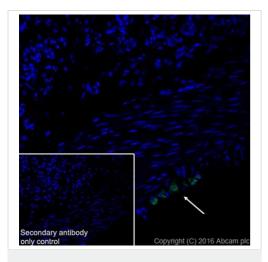
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HuD + HuC antibody
[EPR19098] (ab184267)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling HuC + HuD with ab184267 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasmic and nuclear staining on myenteric ganglia of rat colon is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.



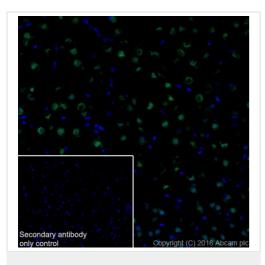
Immunohistochemistry (Frozen sections) - Anti-HuD + HuC antibody [EPR19098] (ab184267)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse stomach tissue labeling HuC + HuD with ab184267 at 1/500 dilution, followed by Goat antirabbit $\lg G$ (Alexa Fluor 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Cytoplasmic and nuclear staining on myenteric ganglia of mouse stomach is observed (white arrow).

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.



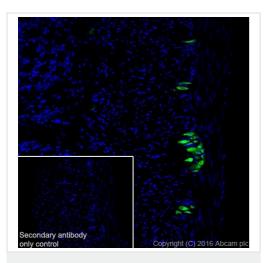
Immunohistochemistry (Frozen sections) - Anti-HuD + HuC antibody [EPR19098] (ab184267)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen rat cerebral cortex tissue labeling HuC + HuD with ab184267 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Cytoplasmic and nuclear staining on neurons of rat cerebral cortex is observed.

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.



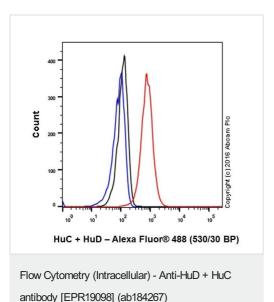
Immunohistochemistry (Frozen sections) - Anti-HuD + HuC antibody [EPR19098] (ab184267)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen rat colon tissue labeling HuC + HuD with ab184267 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

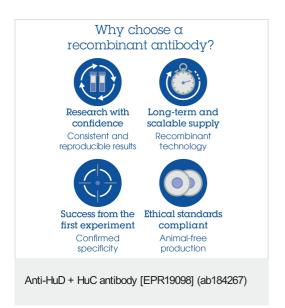
Cytoplasmic and nuclear staining on myenteric ganglia of rat colon is observed.

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.



Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed Neuro-2a (Mouse neuroblastoma cell line) cells labeling HuC + HuDwith ab184267 at 1/500 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor[®] 488) at 1/2000 dilution was used as the secondary antibody.



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