abcam

Product datasheet

Anti-Huntingtin antibody [EPR5526] - BSA and Azide free ab209668

KO VALIDATED Recombinant RabMAb

1 References 12 Images

Overview

Product name	Anti-Huntingtin antibody [EPR5526] - BSA and Azide free
Description	Rabbit monoclonal [EPR5526] to Huntingtin - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-FoFr, IHC-P, WB, ICC/IF, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Huntingtin aa 1-100. The exact sequence is proprietary. Database link: P42858
Positive control	WB: SH-SY5Y, HeLa, HAP1, PC-12 and Neuro-2a whole cell lysates and mouse and rat brain lysates. IHC-P: Human cerebral cortex tissue, Human astrocytoma tissue, Mouse testis and Rat testis tissue. ICC/IF: Neuro-2a and SH-SY5Y cell lines. Flow cytometry: SH-SY5Y cell line. IHC-Fr: Mouse cerebellum tissue
General notes	Ab209668 is the carrier-free version of ab109115. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. ab209668 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* 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.

Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5526
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab209668** in the following tested applications.

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

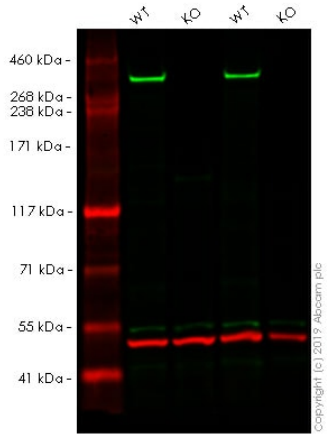
Application	Abreviews	Notes
IHC-FoFr		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 348 kDa (predicted molecular weight: 348 kDa).

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function	May play a role in microtubule-mediated transport or vesicle function.
Tissue specificity	Expressed in the brain cortex (at protein level). Widely expressed with the highest level of expression in the brain (nerve fibers, varicosities, and nerve endings). In the brain, the regions where it can be mainly found are the cerebellar cortex, the neocortex, the striatum, and the hippocampal formation.
Involvement in disease	Defects in HTT are the cause of Huntington disease (HD) [MIM:143100]. HD is an autosomal dominant neurodegenerative disorder characterized by involuntary movements (chorea), general motor impairment, psychiatric disorders and dementia. Onset of the disease occurs usually in the third or fourth decade of life and symptoms progressively worsen leading to death in 10 to 20 years. Onset and clinical course depend on the degree of poly-Gln repeat expansion, longer expansions resulting in earlier onset and more severe clinical manifestations. HD affects 1 in 10,000 individuals of European origin. Neuropathology of Huntington disease displays a distinctive pattern with loss of neurons, especially in the caudate and putamen (striatum).
Sequence similarities	Belongs to the huntingtin family. Contains 10 HEAT repeats.
Domain	The N-terminal Gln-rich and Pro-rich domain has great conformational flexibility and is likely to exist in a fluctuating equilibrium of alpha-helical, random coil, and extended conformations.
Post-translational modifications	Cleaved by apopain downstream of the polyglutamine stretch. The resulting N-terminal fragment is cytotoxic and provokes apoptosis. Forms with expanded polyglutamine expansion are specifically ubiquitinated by SYVN1, which promotes their proteasomal degradation.
Cellular localization	Cytoplasm. Nucleus. The mutant Huntingtin protein colocalizes with AKAP8L in the nuclear matrix of Huntington's disease neurons.

Images



Western blot - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

All lanes : Anti-Huntingtin antibody [EPR5526] (ab109115) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : HTT knockout HeLa cell lysate
Lane 3 : Wild-type HAP1 cell lysate
Lane 4 : HTT knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

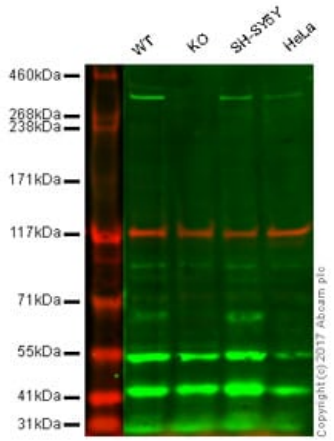
Performed under reducing conditions.

Predicted band size: 348 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab109115).

Lanes 1-4: Merged signal (red and green). Green - ab109115 observed at 348 kDa. Red - loading control ab7291 observed at 50 kDa.

ab109115 Anti-Huntingtin antibody [EPR5526] was shown to specifically react with Huntingtin in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265976 (knockout cell lysate ab256946) was used. Wild-type and Huntingtin knockout samples were subjected to SDS-PAGE. ab109115 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) were incubated overnight at 4°C at 1 in 10000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

All lanes : Anti-Huntingtin antibody [EPR5526] (ab109115) at 1/10000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : HTT knockout HAP1 whole cell lysate
Lane 3 : SH-SY5Y whole cell lysate
Lane 4 : HeLa whole cell lysate

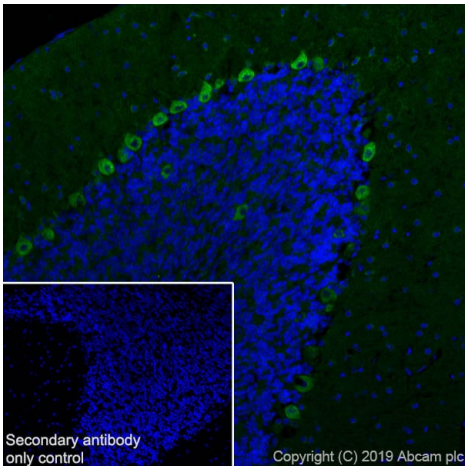
Lysates/proteins at 20 µg per lane.

Predicted band size: 348 kDa

This WB data was generated using the same anti-Huntingtin antibody clone, EPR5526, in a different buffer formulation (cat# ab109115).

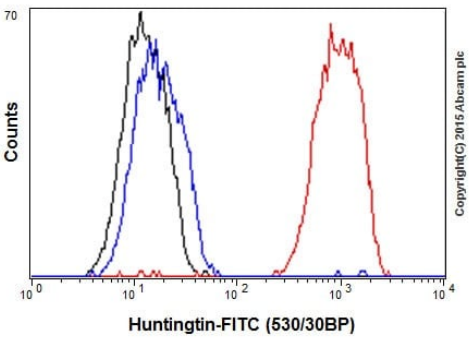
Lanes 1 - 4: Merged signal (red and green). Green - ab109115 observed at 348 kDa. Red - loading control, ab18058, observed at 130 kDa.

ab109115 was shown to specifically recognize HTT in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when HTT knockout samples were exmined. Wild-type and HTT knockout samples were subjected to SDS-PAGE. Unpurified ab109115 and ab18058 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/10,000 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

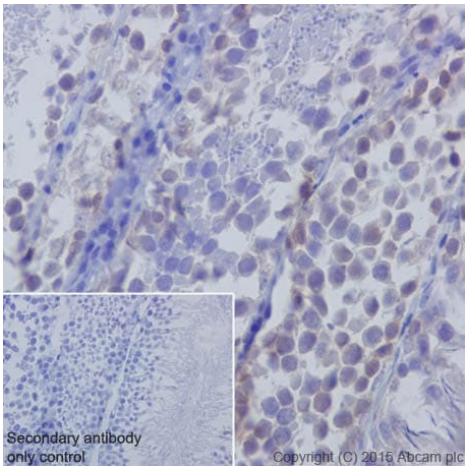
Immunohistochemistry (Frozen) analysis of mouse cerebellum tissue sections labeling Huntingtin with purified ab109115 at 1/100 (13.4 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/1000 (2 µg/ml) was used as the secondary antibody. Sections were fixed with 4% paraformaldehyde and permeabilised with 0.2% Triton X-100. Negative control: PBS instead of the primary antibody. DAPI (blue) was used as nuclear counterstain. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20) was performed.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109115).



Flow Cytometry - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

Flow cytometric analysis of 4% paraformaldehyde-fixed SH-SY5Y (Human neuroblastoma from bone marrow cells) cells labeling Huntingtin with purified ab109115 at 1/250 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109115).

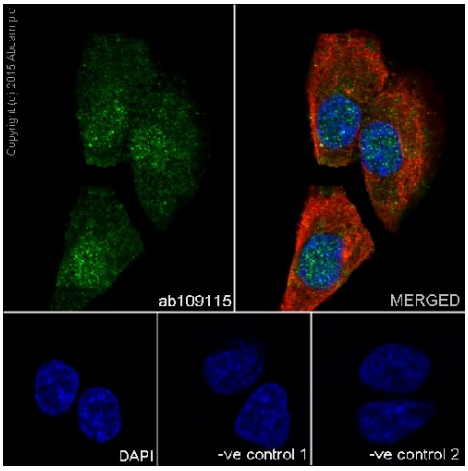


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

Immunohistochemical analysis of paraffin-embedded Rat testis labeling Huntingtin with purified ab109115 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500. Counter stained with Hematoxylin. Weak cytoplasmic staining on spermatogenic cells of rat testis.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109115).



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized

SH-SY5Y (Human neuroblastoma from bone marrow cells) cells labeling Huntingtin with purified ab109115 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

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

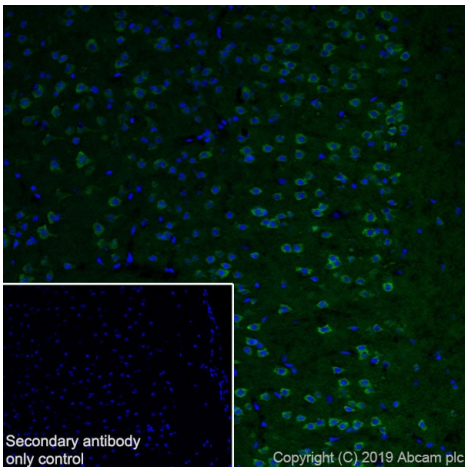
The nuclear counter stain is DAPI (blue).

Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:
1. ab191472 at 1/1000 dilution followed by ab150120 (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

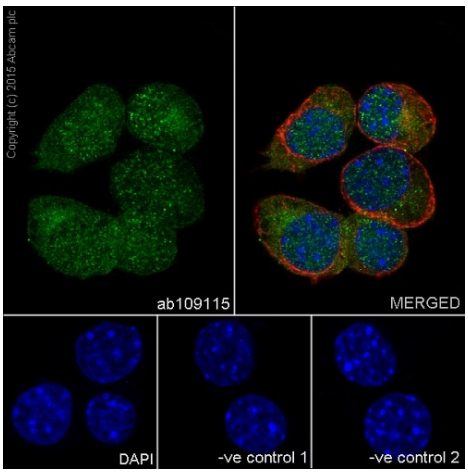
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (ab109115).



Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

Immunohistochemistry (Frozen) analysis of mouse cerebrum tissue sections labeling Huntingtin with purified ab109115 at 1/100 (13.4 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/1000 (2 µg/ml) was used as the secondary antibody. Sections were fixed with 4% paraformaldehyde and permeabilised with 0.2% Triton X-100. Negative control: PBS instead of the primary antibody. DAPI (blue) was used as nuclear counterstain. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20) was performed.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ab109115).



Immunocytochemistry/ Immunofluorescence - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized

Neuro-2a (Mouse neuroblastoma cells) cells labeling Huntingtin with purified ab109115 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

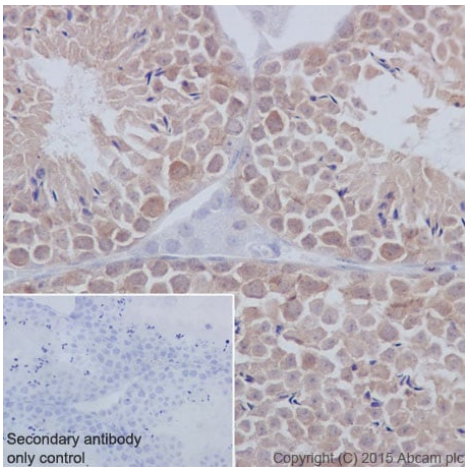
Confocal image showing nuclear and cytoplasmic staining on Neuro-2a cell line.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:
1. ab191472 at 1/1000 dilution followed by ab150120 (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/1000 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109115).

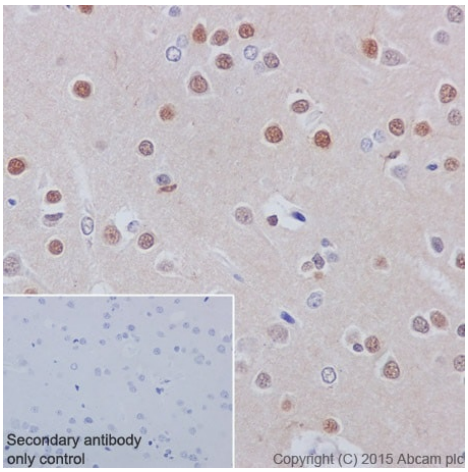


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

Immunohistochemical analysis of paraffin-embedded Mouse testis labeling Huntingtin with purified ab109115 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500. Counter stained with Hematoxylin. Cytoplasmic staining on spermatogenic cells of mouse testis.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109115).

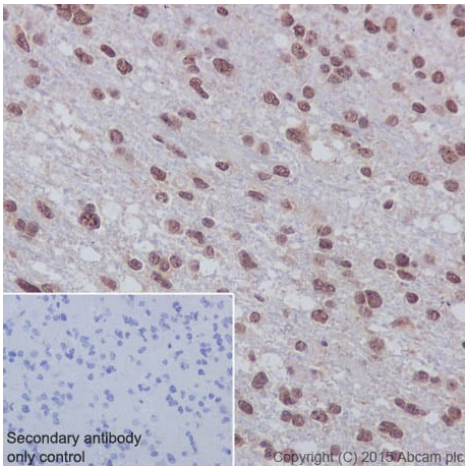


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

Immunohistochemical analysis of paraffin-embedded Human cerebral cortex tissue labeling Huntingtin with purified ab109115 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500. Counter stained with Hematoxylin. Nuclear staining on neuron of human cerebral cortex was observed.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109115).

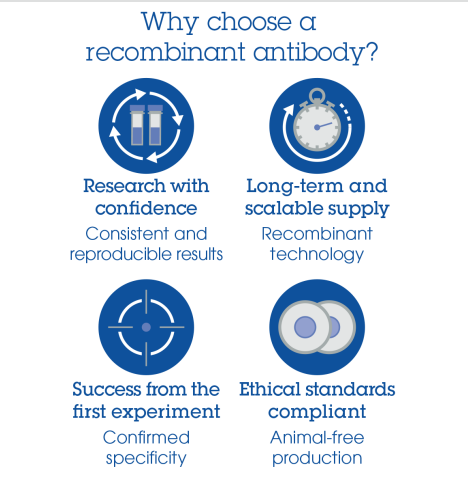


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

This IHC data was generated using the same anti-Huntingtin antibody clone, EPR5526, in a different buffer formulation (cat# ab109115).

Immunohistochemical analysis of paraffin-embedded Human astrocytoma labeling Huntingtin with purified ab109115 at 1/100 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500. Counter stained with Hematoxylin. Nuclear staining on cancer cells of astrocytoma.

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-Huntingtin antibody [EPR5526] - BSA and Azide free (ab209668)

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