abcam

Product datasheet

Anti-Rad21 antibody [EPR22506-15] - ChIP Grade ab217678

Recombinant RabMAb

2 References 14 Images

Overview

Product name	Anti-Rad21 antibody [EPR22506-15] - ChIP Grade
Description	Rabbit monoclonal [EPR22506-15] to Rad21 - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, ChIP, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human heart and fetal kidney tissue lysate. Rat brain and mouse brain and heart tissue lysate. HeLa, MCF7, Raw264.7, NIH/3T3 and C6 cell lysate. IHC-P: Human breast carcinoma, stomach, mouse stomach and rat colon tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow: HeLa and NIH/3T3 cells. IP: HeLa whole cell lysate.
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	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR22506-15
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab217678 in the following tested applications.

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

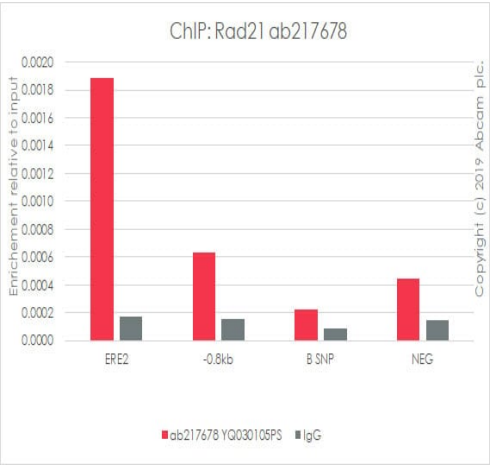
Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
WB		1/1000. Predicted molecular weight: 72 kDa.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.
ICC/IF		1/100.
ChIP		Use at an assay dependent concentration. 5 µg
IP		1/30.

Target

Function	Cleavable component of the cohesin complex, involved in chromosome cohesion during cell cycle, in DNA repair, and in apoptosis. The cohesin complex is required for the cohesion of sister chromatids after DNA replication. The cohesin complex apparently forms a large proteinaceous ring within which sister chromatids can be trapped. At metaphase-anaphase transition, this protein is cleaved by separase/ESPL1 and dissociates from chromatin, allowing sister chromatids to segregate. The cohesin complex may also play a role in spindle pole assembly during mitosis. Also plays a role in apoptosis, via its cleavage by caspase-3/CASP3 or caspase-7/CASP7 during early steps of apoptosis: the C-terminal 64 kDa cleavage product may act as a nuclear signal to initiate cytoplasmic events involved in the apoptotic pathway.
Sequence similarities	Belongs to the rad21 family.
Domain	The C-terminal part associates with the head of SMC1A, while the N-terminal part binds to the head of SMC3.
Post-translational modifications	Cleaved by separase/ESPL1 at the onset of anaphase. Cleaved by caspase-3 and caspase-7 at the beginning of apoptosis. The cleavage by ESPL1 and caspase-3 take place at different sites. Phosphorylated; becomes hyperphosphorylated in M phase of cell cycle. The large dissociation of cohesin from chromosome arms during prophase may be partly due to its phosphorylation by PLK.
Cellular localization	Nucleus. Chromosome. Chromosome > centromere. Associates with chromatin. Before prophase it is scattered along chromosome arms. During prophase, most of cohesin complexes dissociate from chromatin probably because of phosphorylation by PLK, except at centromeres, where

cohesin complexes remain. At anaphase, it is cleaved by separase/ESPL1, leading to the dissociation of the complex from chromosomes, allowing chromosome separation. Once cleaved by caspase-3, the C-terminal 64 kDa cleavage product translocates to the cytoplasm, where it may trigger apoptosis.

Images



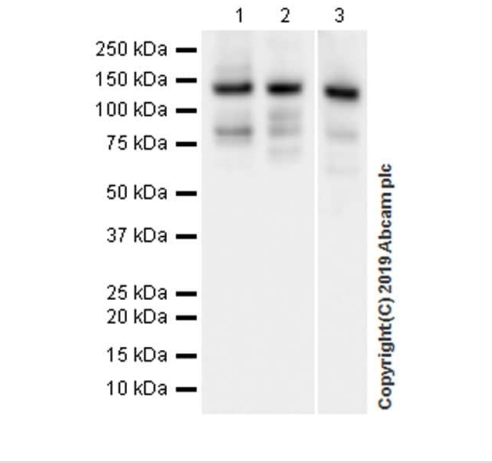
ChIP - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

Chromatin was prepared from MCF7 cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab217678 (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.

The observed enrichment profile of Rad21 binding is consistent with what has been described in the literature (PMID: 23145106)



Western blot - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

All lanes : Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678) at 1/1000 dilution

Lane 1 : RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage), whole cell lysate
Lane 2 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 3 : C6 (rat glial tumor glial cell), whole cell lysate whole cell lysate

Lysates/proteins at 20 µg per lane.

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

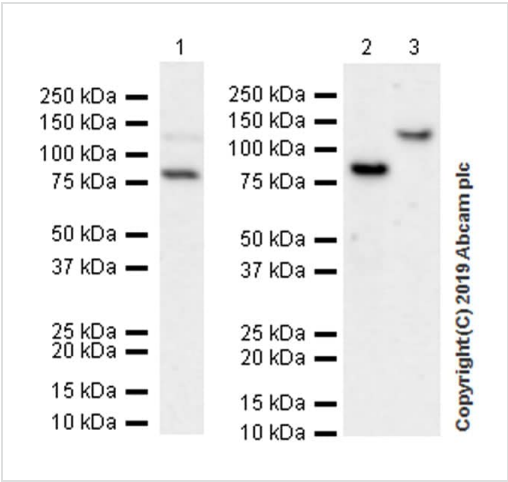
Predicted band size: 72 kDa
Observed band size: 130-75 kDa

Full length Rad21 is approximately 130kDa, cleaved Rad21 is approximately 75 kDa. The molecular weight observed are

consistent with what has been described in the literature (PMID: 12417729, PMID 21876002).

Exposure time: 7.7 seconds

Blocking/diluting buffer and concentration: 5% NFDM/TBST



Western blot - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

All lanes : Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678) at 1/1000 dilution

Lane 1 : Rat Brain tissue lysate
Lane 2 : Mouse Brain tissue lysate
Lane 3 : Mouse heart tissue lysate

Lysates/proteins at 20 µg per lane.

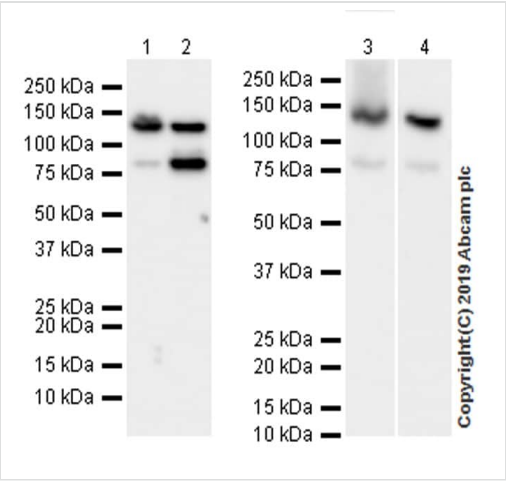
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 72 kDa
Observed band size: 130-75 kDa

Full length Rad21 is approximately 130kDa, cleaved Rad21 is approximately 75 kDa. The molecular weight observed are consistent with what has been described in the literature (PMID: 12417729, PMID 21876002).

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

Exposure time: 3 minutes.



Western blot - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

All lanes : Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678) at 1/1000 dilution

Lane 1 : Human heart tissue lysate
Lane 2 : Human fetal kidney tissue lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 4 : MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
Lanes 1-2 : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/1000 dilution
Lanes 3-4 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 72 kDa
Observed band size: 130-75 kDa

Full length Rad21 is approximately 130 kDa, cleaved Rad21 is approximately 75 kDa. The molecular weight observed are consistent with what has been described in the literature (PMID: 12417729, PMID 21876002).

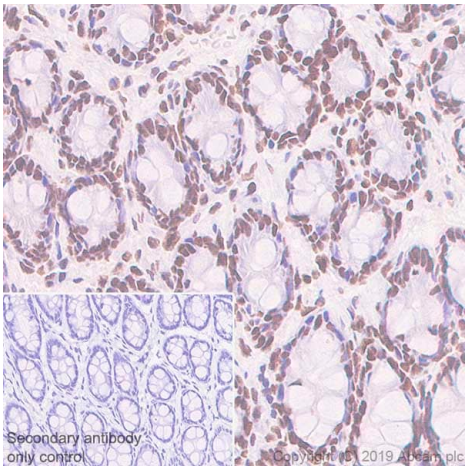
Blocking/Diluting buffer and concentration: 5% NFDM/TBST

Exposure times:

Lanes1-2: 37 seconds

Lane 3: 5.5 seconds

Lane 4: 48 seconds

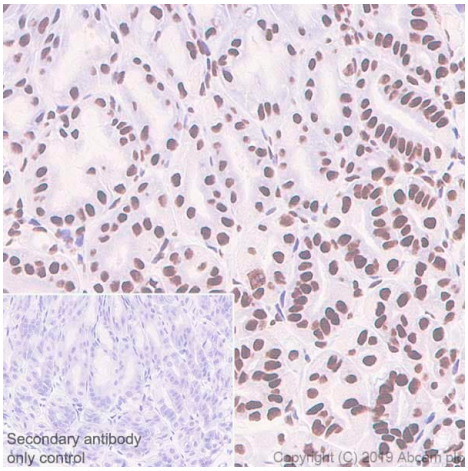


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling Rad21 using ab217678 at 1/1000 dilution for 30 minutes at room temperature, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on rat colon (PMID: 25575569) is observed. Counterstained with Hematoxylin. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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

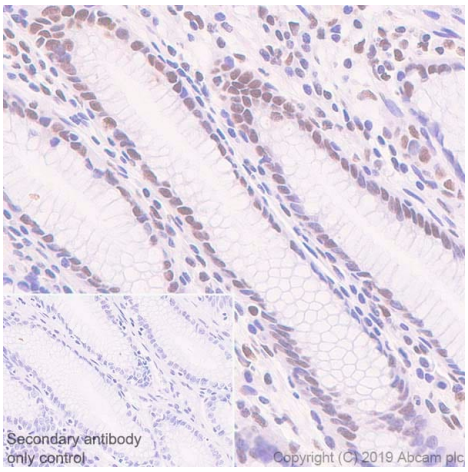


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

Immunohistochemical analysis of paraffin-embedded mouse stomach tissue labeling Rad21 using ab217678 at 1/1000 dilution for 30 minutes at room temperature, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on mouse stomach (PMID: 25575569) is observed. Counterstained with Hematoxylin. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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

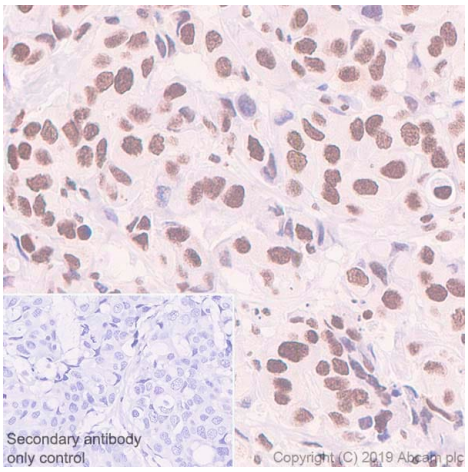


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling Rad21 using ab217678 at 1/1000 dilution for 30 minutes at room temperature, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human stomach (PMID: 25575569) is observed. Counterstained with Hematoxylin. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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

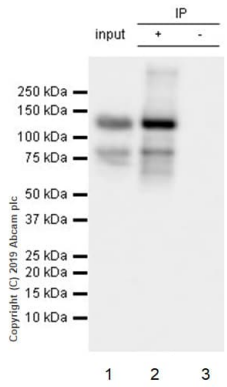


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue labeling Rad21 using ab217678 at 1/1000 dilution for 30 minutes at room temperature, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on the human breast carcinoma (PMID: 21255398) is observed. Counterstained with Hematoxylin. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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



Immunoprecipitation - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

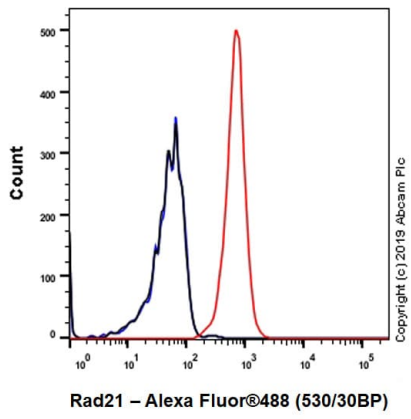
Rad21 was immunoprecipitated from 0.35mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate with ab217678 at 1/30 dilution. Western blot was performed on the immunoprecipitate using ab217678 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (**ab131366**) was used as the secondary antibody at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10µg (input)
Lane 2: ab217678 IP in HeLa whole cell lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab217678 in HeLa whole cell lysate.

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

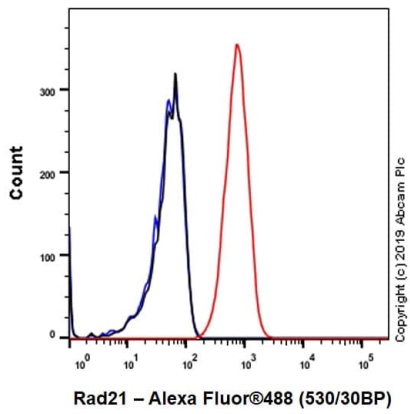
Exposure time: 3 seconds.

Faint band below 75kDa in lane 2 could be Rad21 cleavage product as described in the literature (PMID: 28854249).



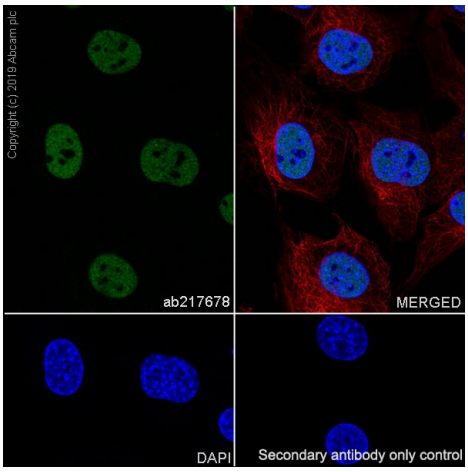
Flow Cytometry (Intracellular) - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed NIH/3T3 (mouse embryonic fibroblast) cell line labeling Rad21 using ab217678 at 1/500 dilution (red) compared with a Rabbit monoclonal IgG (**ab172730**, Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

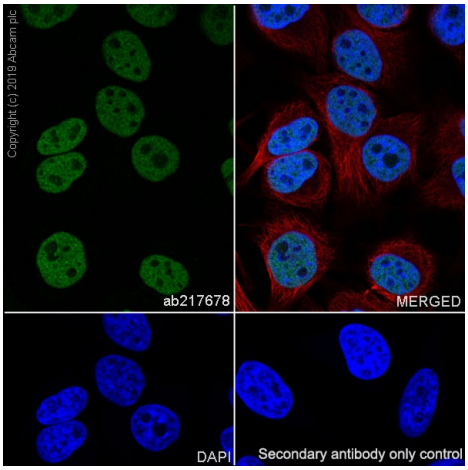
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (human cervix adenocarcinoma epithelial cell) cell line labeling Rad21 using ab217678 at 1/500 dilution (red) compared with a Rabbit monoclonal IgG (**ab172730**, Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labeling Rad21 with ab217678 at 1/100 dilution, followed by an AlexaFluor®488 Goat anti-Rabbit secondary antibody (**ab150077**) at 1/1000 dilution (green). Confocal image showing nuclear staining in NIH/3T3 cell line is observed. The nuvlear counterstain is DAPI (Blue). Tubulin is counterstained using Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is an AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Rad21 with ab217678 at 1/100 dilution, followed by an AlexaFluor®488 Goat anti-Rabbit secondary antibody (**ab150077**) at 1/1000 dilution (green). Confocal image showing nuclear staining in HeLa cell line is observed. The nuvlear counterstain is DAPI (Blue). Tubulin is counterstained using Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is an AlexaFluor®488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution.

Anti-Rad21 antibody [EPR22506-15] - ChIP Grade (ab217678)

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