abcam

Product datasheet

Anti-MeCP2 antibody [EPR23201-3] ab253197

KO VALIDATED Recombinant RabMAb

4 Abreviews 2 References 18 Images

Overview

Product name	Anti-MeCP2 antibody [EPR23201-3]
Description	Rabbit monoclonal [EPR23201-3] to MeCP2
Host species	Rabbit
Specificity	This antibody did not work in IHC in human brain samples.
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, WB, IHC-P, ICC/IF **Unsuitable for:** ChIP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HepG2, SH-SY5Y, Neuro-2a and HEK-293T cell lysates; Mouse brain and hippocampus lysates; Rat brain and liver lysates; Human brain, hippocampus and liver lysates. IHC-P: Mouse lung and testis tissues; Rat cerebrum and lung tissues; Human kidney and astrocytoma tissues. ICC/IF: SH-SY5Y and Neuro-2a cells. Flow Cyt (intra): SH-SY5Y and Neuro-2a cells.
General notes	This antibody did not work in IHC in human brain samples. 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	EPR23201-3
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab253197 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)		1/600.
IP		Use at an assay dependent concentration.
WB	★★★☆☆ (2)	1/1000. Predicted molecular weight: 52 kDa.
IHC-P		1/4000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. This antibody did not work in IHC in human brain samples.
ICC/IF	★★★★★ (2)	1/500.

Application notes Is unsuitable for ChIP.

Target

Function Chromosomal protein that binds to methylated DNA. It can bind specifically to a single methyl-CpG pair. It is not influenced by sequences flanking the methyl-CpGs. Mediates transcriptional repression through interaction with histone deacetylase and the corepressor SIN3A.

Tissue specificity Present in all adult somatic tissues tested.

Involvement in disease Defects in MECP2 may be a cause of Angelman syndrome (AS) [MIM:105830]; also known as happy puppet syndrome. AS is a neurodevelopmental disorder characterized by severe mental retardation, absent speech, ataxia, sociable affect and dysmorphic facial features. AS and Rett syndrome have overlapping clinical features.
Defects in MECP2 are the cause of mental retardation syndromic X-linked type 13 (MRXS13) [MIM:300055]. Mental retardation is a mental disorder characterized by significantly sub-average general intellectual functioning associated with impairments in adaptative behavior and manifested during the developmental period. MRXS13 patients manifest mental retardation associated with other variable features such as spasticity, episodes of manic depressive psychosis, increased tone and macroorchidism.
Defects in MECP2 are the cause of Rett syndrome (RTT) [MIM:312750]. RTT is an X-linked dominant disease, it is a progressive neurologic developmental disorder and one of the most common causes of mental retardation in females. Patients appear to develop normally until 6 to 18 months of age, then gradually lose speech and purposeful hand movements and develop microcephaly, seizures, autism, ataxia, intermittent hyperventilation, and stereotypic hand

movements. After initial regression, the condition stabilizes and patients usually survive into adulthood.
Defects in MECP2 may be the cause of susceptibility autism X-linked type 3 (AUTSX3) [MIM:300496]. AUTSX3 is a pervasive developmental disorder (PDD), prototypically characterized by impairments in reciprocal social interaction and communication, restricted and stereotyped patterns of interests and activities, and the presence of developmental abnormalities by 3 years of age.
Defects in MECP2 are the cause of encephalopathy neonatal severe due to MECP2 mutations (ENS-MECP2) [MIM:300673]. Note=The MECP2 gene is mutated in Rett syndrome, a severe neurodevelopmental disorder that almost always occurs in females. Although it was first thought that MECP2 mutations causing Rett syndrome were lethal in males, later reports identified a severe neonatal encephalopathy in surviving male sibs of patients with Rett syndrome. Additional reports have confirmed a severe phenotype in males with Rett syndrome-associated MECP2 mutations.
Defects in MECP2 are the cause of mental retardation syndromic X-linked Lubs type (MRXSL) [MIM:300260]. Mental retardation is characterized by significantly below average general intellectual functioning associated with impairments in adaptative behavior and manifested during the developmental period. MRXSL patients manifest mental retardation associated with variable features. They include swallowing dysfunction and gastroesophageal reflux with secondary recurrent respiratory infections, hypotonia, mild myopathy and characteristic facies such as downslanting palpebral fissures, hypertelorism and a short nose with a low nasal bridge. Note=Increased dosage of MECP2 due to gene duplication appears to be responsible for the mental retardation phenotype.

Sequence similarities

Contains 2 A.T hook DNA-binding domains.
Contains 1 MBD (methyl-CpG-binding) domain.

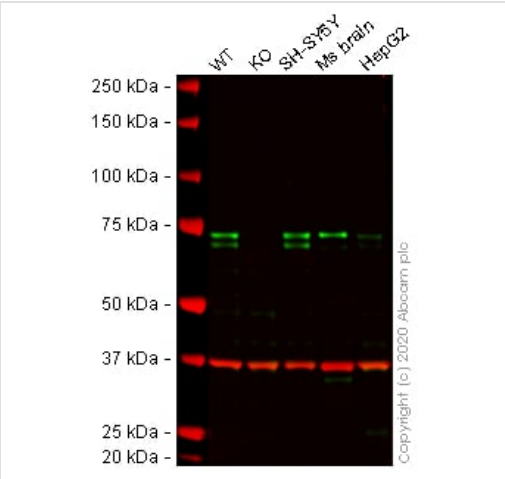
Post-translational modifications

Phosphorylated on Ser-423 in brain upon synaptic activity, which attenuates its repressor activity and seems to regulate dendritic growth and spine maturation.

Cellular localization

Nucleus. Colocalized with methyl-CpG in the genome.

Images



Western blot - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

All lanes : Anti-MeCP2 antibody [EPR23201-3] (ab253197) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : MECP2 knockout HAP1 cell lysate
Lane 3 : SH-SY5Y cell lysate
Lane 4 : Mouse Brain cell lysate
Lane 5 : HepG2 cell lysate

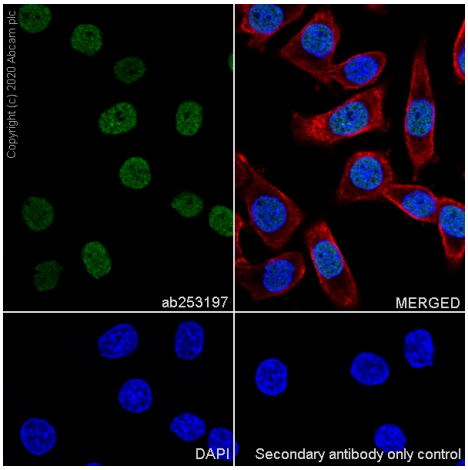
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 52 kDa
Observed band size: 68-70 kDa

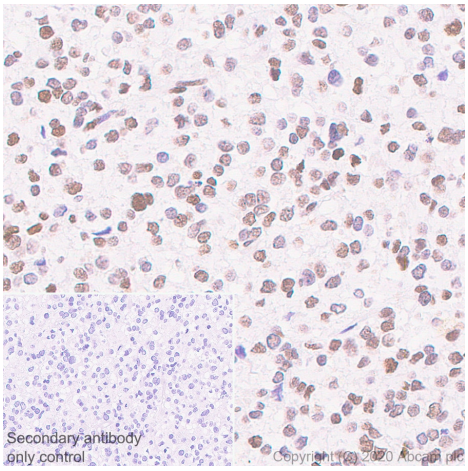
Lanes 1 - 5: Merged signal (red and green). Green - ab253197 observed at 68-70 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab253197 was shown to react with MeCP2 in wild-type HAP1 cells in western blot. Loss of signal was observed when MECP2 knockout sample was used. Wild-type and MECP2 knockout HAP1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBS-T (0.1% Tween®) before incubation with ab253197 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized SH-SY5Y cells labelling MeCP2 with ab253197 at 1/500 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2 ug/ml dilution (Green). Confocal image showing nuclear staining in SH-SY5Y cells. Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5 dilution (Red). The Nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1000 2 ug/ml dilution.

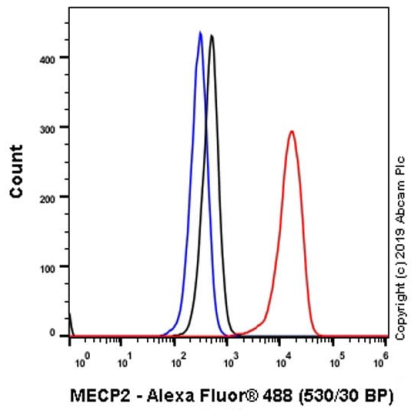


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Immunohistochemical analysis of paraffin-embedded Human astrocytoma tissue labeling MeCP2 with ab253197 at 1/4000 dilution (0.158 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human astrocytoma (PMID: 11809720). The section was incubated with ab253197 for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

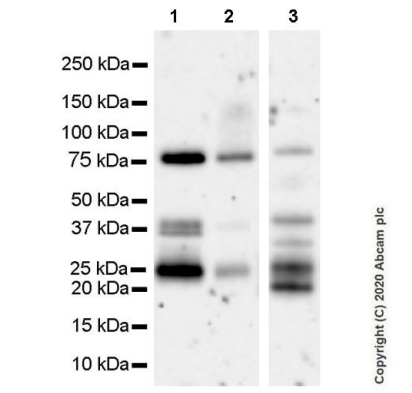
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Flow Cytometry (Intracellular) - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized SH-SY5Y (Human neuroblastoma epithelial cell) cells labelling MeCP2 with ab253197 at 1/600 dilution (0.1ug) (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). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

All lanes : Anti-MeCP2 antibody [EPR23201-3] (ab253197) at 1/1000 dilution

Lane 1 : Human brain tissue lysate
Lane 2 : Human hippocampus tissue lysate
Lane 3 : Human liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

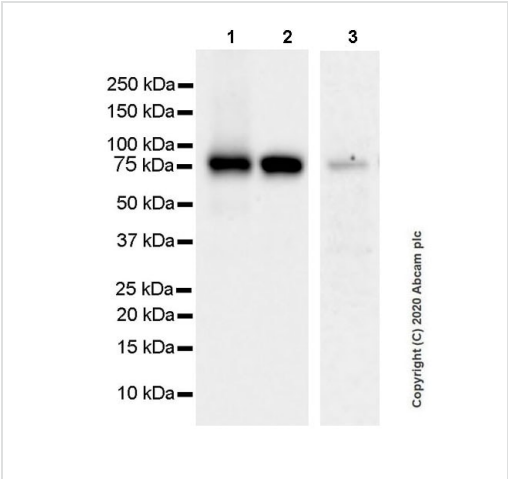
Predicted band size: 52 kDa

Observed band size: 75 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST
This blot was developed using a higher sensitivity ECL substrate.

The lower bands below 75kDa should be degraded MeCP2 fragments(PMID:31601272), freshly prepared lysates were recommended to use in avoid of target protein degradation.

Exposure time: 3 minutes



Western blot - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

All lanes : Anti-MeCP2 antibody [EPR23201-3] (ab253197) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate
Lane 2 : Mouse hippocampus tissue lysate
Lane 3 : Neuro-2a (mouse neuroblastoma neuroblast), whole cell lysate

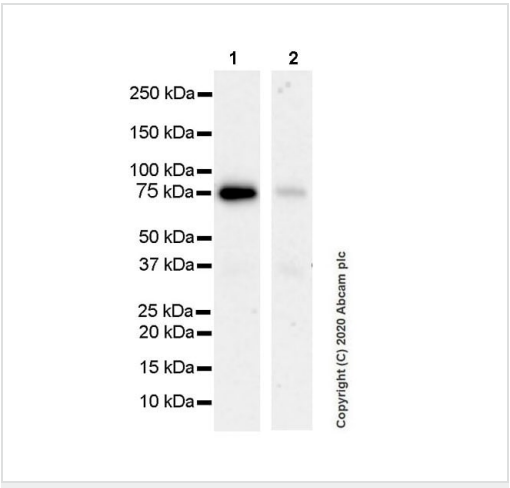
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 52 kDa
Observed band size: 75 kDa

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

Exposure time: Lane1-2: 30 seconds Lane 3: 3 minutes



Western blot - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

All lanes : Anti-MeCP2 antibody [EPR23201-3] (ab253197) at 1/1000 dilution

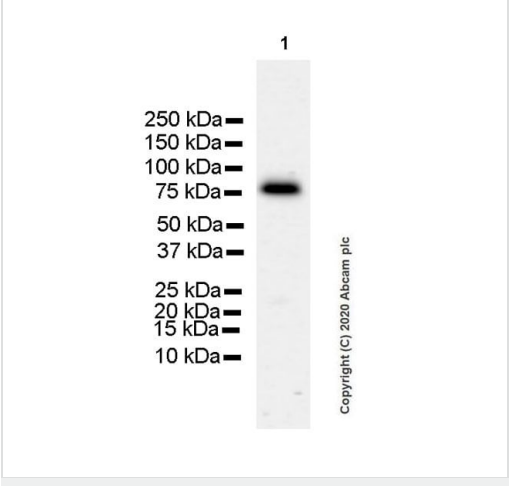
Lane 1 : Rat brain tissue lysate
Lane 2 : Rat liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 52 kDa
Observed band size: 75 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST
Exposure time: 3 minutes



Western blot - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Anti-MeCP2 antibody [EPR23201-3] (ab253197) at 1/1000 dilution + SH-SY5Y (human neuroblastoma epithelial cell), whole cell lysate at 20 µg

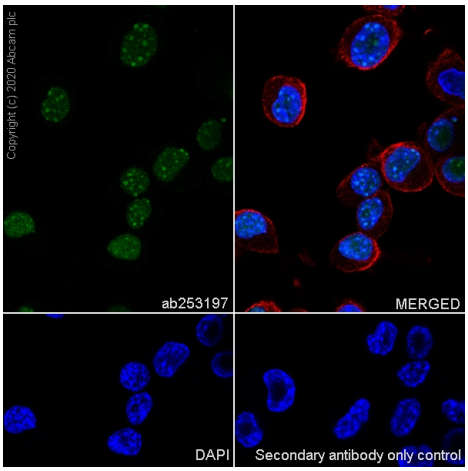
Secondary
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 52 kDa
Observed band size: 75 kDa

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

This antibody specifically recognizes MeCP2 protein at around 75kDa (PMID: 27995568, PMID: 28394263)

Exposure time: 114 seconds



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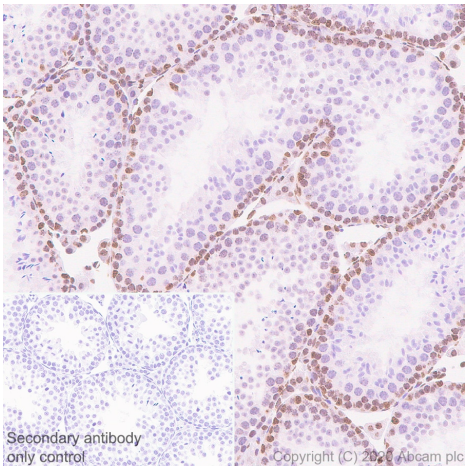
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Neuro-2a cells labelling MeCP2 with ab253197 at 1/500 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2 ug/ml dilution (Green). Confocal image showing nuclear staining in Neuro-2a cells. Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5 dilution (Red). The Nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1000 2 ug/ml dilution.

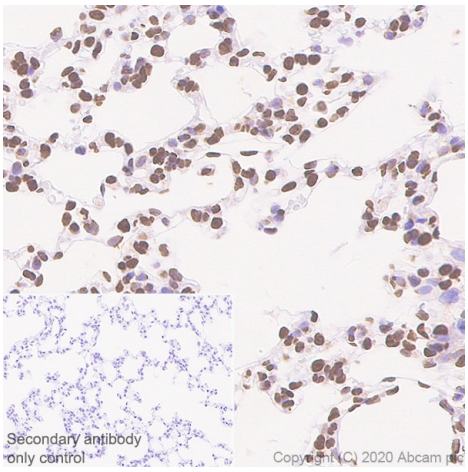


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labeling MeCP2 with ab253197 at 1/4000 dilution (0.158 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on mouse testis (PMID: 11809720). The section was incubated with ab253197 for 10 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

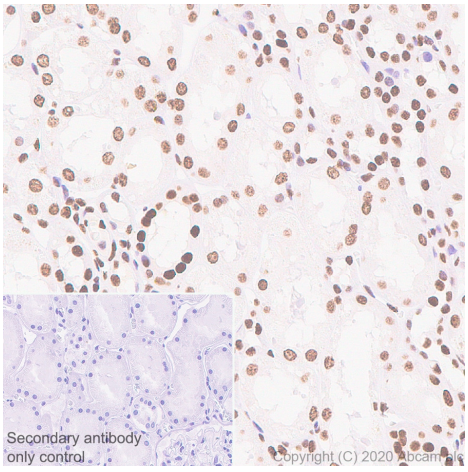


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Immunohistochemical analysis of paraffin-embedded Rat lung tissue labeling MeCP2 with ab253197 at 1/4000 dilution (0.158 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on rat lung (PMID: 11809720). The section was incubated with ab253197 for 10 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

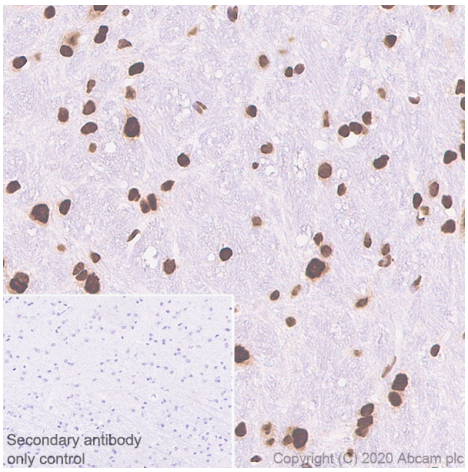


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling MeCP2 with ab253197 at 1/4000 dilution (0.158 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human kidney (PMID: 11809720). The section was incubated with ab253197 for 10 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

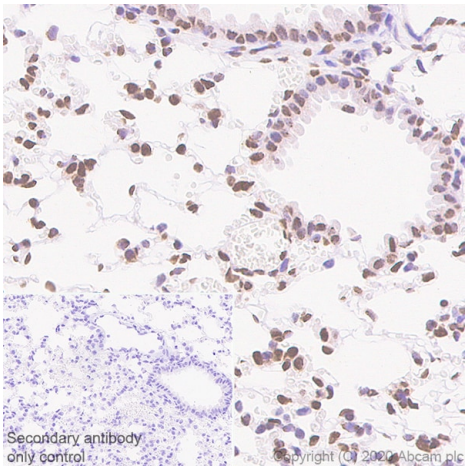


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling MeCP2 with ab253197 at 1/4000 dilution (0.158 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on rat cerebrum (PMID: 11809720). The section was incubated with ab253197 for 10 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

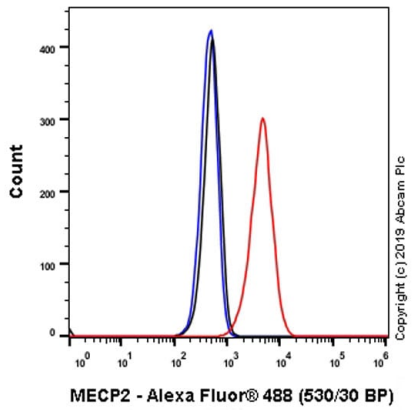


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Immunohistochemical analysis of paraffin-embedded Mouse lung tissue labeling MeCP2 with ab253197 at 1/4000 dilution (0.158 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on mouse lung (PMID: 11809720). The section was incubated with ab253197 for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

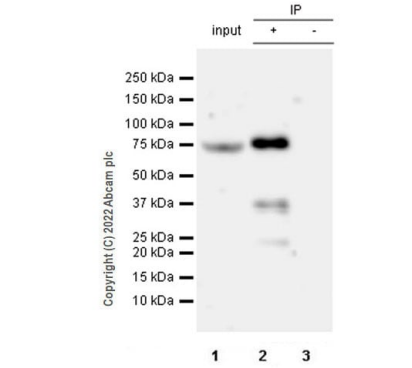
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Flow Cytometry (Intracellular) - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Neuro-2a (Mouse neuroblastoma neuroblast) cells labelling MeCP2 with ab253197 at 1/600 dilution (0.1ug) (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). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

MeCP2 was immunoprecipitated from 0.35 mg of 293T (human embryonic kidney epithelial cell), whole cell lysate with ab253197 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab253197 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution.

Lane 1: 293T whole cell lysate 10 µg (Input).

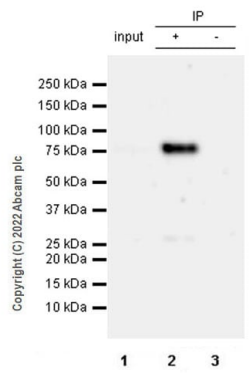
Lane 2: ab253197 IP in 293T whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab253197 in 293T whole cell lysate

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

Exposure time: 58 seconds.

The lower bands below 75kDa could be degraded MeCP2 fragments (PMID:31601272).



Immunoprecipitation - Anti-MeCP2 antibody [EPR23201-3] (ab253197)

MeCP2 was immunoprecipitated from 0.35 mg of Neuro-2a (mouse neuroblastoma neuroblast), whole cell lysate with ab253197 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab253197 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution.

Lane 1: Neuro-2a whole cell lysate 10 µg (Input).

Lane 2: ab253197 IP in Neuro-2a whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab253197 in Neuro-2a whole cell lysate

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

Exposure time: 58 seconds.

Anti-MeCP2 antibody [EPR23201-3] (ab253197)

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