abcam

Product datasheet

Anti-p53R2 antibody [EPR8816] ab154194

KO VALIDATED Recombinant RabMAb

6 References 18 Images

Overview

Product name	Anti-p53R2 antibody [EPR8816]
Description	Rabbit monoclonal [EPR8816] to p53R2
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: MCF7, HCT116, Human skeletal muscle, and SW480 lysates. IHC-P: human breast carcinoma tissue. ICC/IF: HeLa cells. Flow Cyt (intra): MCF7 cells. IP: MCF7 cells.
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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20ºC.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR8816
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab154194 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
WB		1/1000 - 1/10000. Predicted molecular weight: 40 kDa.
IHC-P		1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. For unpurified use at 1/50 - 1/100
ICC/IF		1/50.
IP		1/10 - 1/100.
Flow Cyt (Intra)		1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function Plays a pivotal role in cell survival by repairing damaged DNA in a p53/TP53-dependent manner. Supplies deoxyribonucleotides for DNA repair in cells arrested at G1 or G2. Contains an iron-tyrosyl free radical center required for catalysis. Forms an active ribonucleotide reductase (RNR) complex with RRM1 which is expressed both in resting and proliferating cells in response to DNA damage.

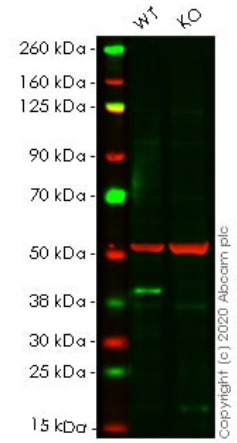
Tissue specificity Widely expressed at a high level in skeletal muscle and at a weak level in thymus. Expressed in epithelial dysplasias and squamous cell carcinoma.

Pathway Genetic information processing; DNA replication.

Involvement in disease Defects in RRM2B are the cause of mitochondrial DNA depletion syndrome type 8A (MTDPS8A) [MIM:612075]. A disorder due to mitochondrial dysfunction characterized by various combinations of neonatal hypotonia, neurological deterioration, respiratory distress, lactic acidosis, and renal tubulopathy.
Defects in RRM2B are the cause of mitochondrial DNA depletion syndrome type 8B (MTDPS8B) [MIM:612075]. A disease due to mitochondrial dysfunction and characterized by ophthalmoplegia, ptosis, gastrointestinal dysmotility, cachexia, peripheral neuropathy.
Defects in RRM2B are the cause of progressive external ophthalmoplegia with mitochondrial DNA deletions autosomal dominant type 5 (PEOA5) [MIM:613077]. A disorder characterized by progressive weakness of ocular muscles and levator muscle of the upper eyelid. In a minority of cases, it is associated with skeletal myopathy, which predominantly involves axial or proximal muscles and which causes abnormal fatigability and even permanent muscle weakness. Ragged-red fibers and atrophy are found on muscle biopsy. A large proportion of chronic ophthalmoplegias are associated with other symptoms, leading to a multisystemic pattern of this disease. Additional symptoms are variable, and may include cataracts, hearing loss, sensory axonal neuropathy, ataxia, depression, hypogonadism, and parkinsonism.

Sequence similarities	Belongs to the ribonucleoside diphosphate reductase small chain family.
Cellular localization	Cytoplasm. Nucleus. Translocates from cytoplasm to nucleus in response to DNA damage.

Images



Western blot - Anti-p53R2 antibody [EPR8816] (ab154194)

All lanes : Anti-p53R2 antibody [EPR8816] (ab154194) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RRM2B knockout HeLa cell lysate

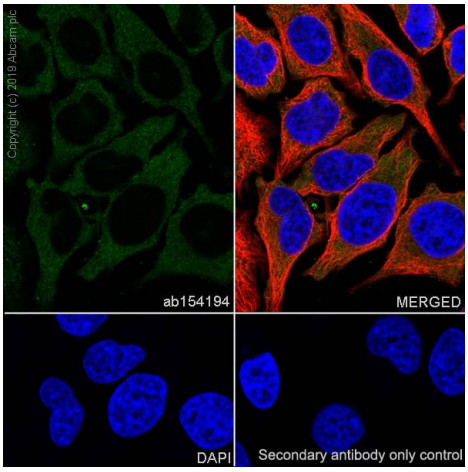
Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

Predicted band size: 40 kDa
Observed band size: 40 kDa

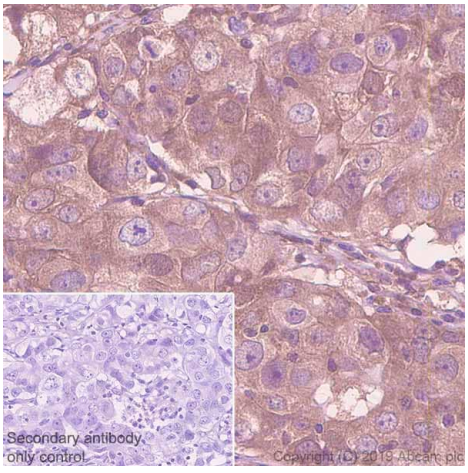
Lanes 1- 2: Merged signal (red and green). Green - ab154194 observed at 40 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab154194 was shown to react with p53R2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab261769** (knockout cell lysate **ab257215**) was used. Wild-type HeLa and RRM2B knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab154194 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 1000 dilution and a 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.



Immunocytochemistry/ Immunofluorescence - Anti-p53R2 antibody [EPR8816] (ab154194)

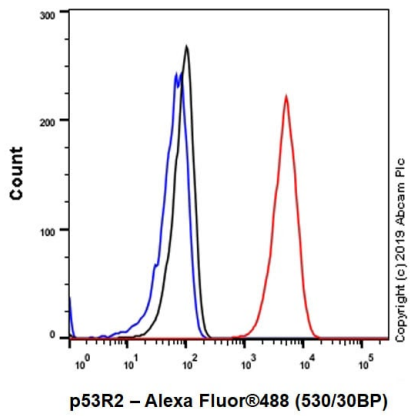
Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling p53R2 with purified ab154194 at 1/50 dilution (2.2 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53R2 antibody [EPR8816] (ab154194)

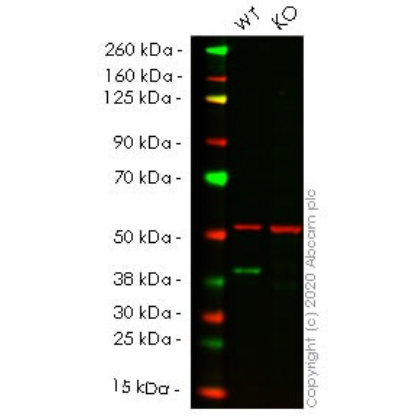
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling p53R2 with purified ab154194 at 1/500 dilution (0.22 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody.

Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Flow Cytometry (Intracellular) - Anti-p53R2 antibody [EPR8816] (ab154194)

Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling p53R2 with purified ab154194 at 1/20 dilution (10µg/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-p53R2 antibody [EPR8816] (ab154194)

All lanes : Anti-p53R2 antibody [EPR8816] (ab154194) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : RRM2B knockout HCT116 cell lysate

Lysates/proteins at 20 µg per lane.

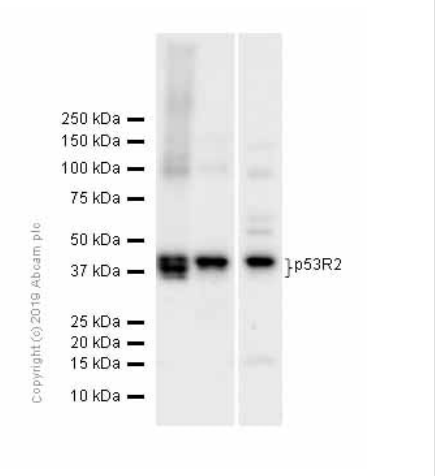
Performed under reducing conditions.

Predicted band size: 40 kDa
Observed band size: 40 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab154194 observed at 40 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab154194 was shown to react with p53R2 in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line **ab266897** (knockout cell lysate **ab257216**) was used. Wild-Type HCT116 and RRM2B knockout HCT116 cell lysates were

subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab154194 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 1000 dilution and a 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-p53R2 antibody [EPR8816] (ab154194)

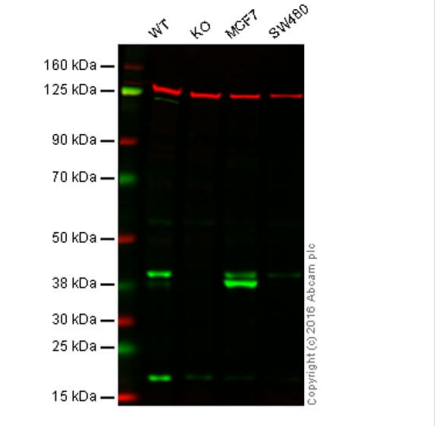
All lanes : Anti-p53R2 antibody [EPR8816] (ab154194) at 1/1000 dilution (Purified)

Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : Human skeletal muscle lysates
Lane 3 : SW480 (Human colorectal adenocarcinoma epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 40 kDa
Observed band size: 41 kDa



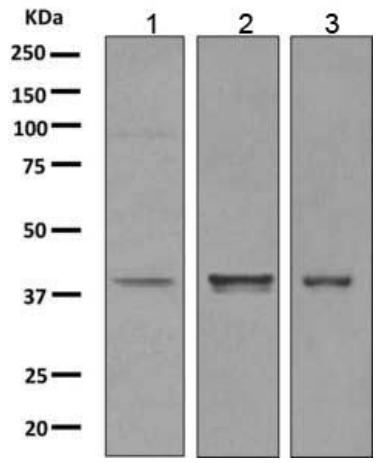
Western blot - Anti-p53R2 antibody [EPR8816] (ab154194)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: p53R2 knockout HAP1 cell lysate (20 µg)
Lane 3: MCF7 cell lysate (20 µg)
Lane 4: SW480 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab154194 observed at 40 kDa. Red - loading control, **ab18058**, observed at 124 kDa.

Unpurified ab154194 was shown to recognize p53R2 when p53R2 knockout samples were used, along with additional cross-reactive bands. Wild-type and p53R2 knockout samples were subjected to SDS-PAGE. ab154194 and **ab18058** (loading control to Vinculin) were both diluted 1/1000 and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-p53R2 antibody [EPR8816] (ab154194)

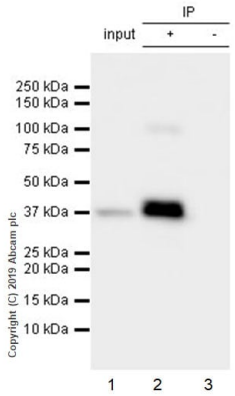
All lanes : Anti-p53R2 antibody [EPR8816] (ab154194) at 1/1000 dilution ((unpurified))

Lane 1 : Human fetal muscle lysate
Lane 2 : MCF7 cell lysate
Lane 3 : SW480 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-rabbit HRP at 1/2000 dilution

Predicted band size: 40 kDa



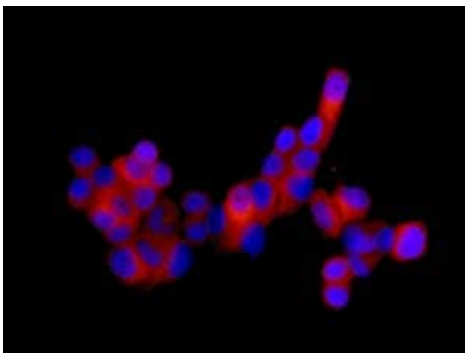
Immunoprecipitation - Anti-p53R2 antibody [EPR8816] (ab154194)

ab154194 (purified) at 1/20 dilution (0.5ug) immunoprecipitating p53R2 in MCF7 whole cell lysate.

Lane 1 (input): MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate 10ug
Lane 2 (+): ab154194 & MCF7 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab154194 in MCF7 whole cell lysate

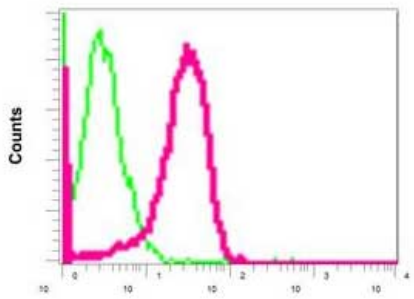
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



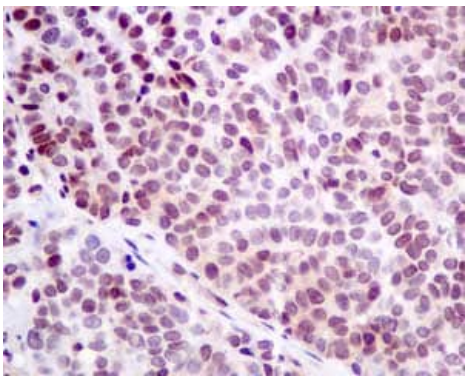
Immunocytochemistry/ Immunofluorescence - Anti-p53R2 antibody [EPR8816] (ab154194)

Immunofluorescent staining of MCF7 cells labeling p53R2 with unpurified ab154194 at 1/250 dilution.



Flow Cytometry (Intracellular) - Anti-p53R2 antibody [EPR8816] (ab154194)

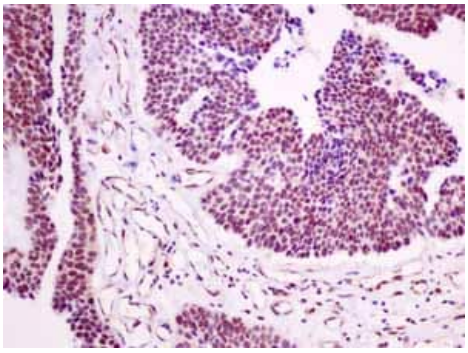
Intracellular flow cytometric analysis of permeabilized MCF7 cells labeling p53R2 with unpurifiedab154194 at 1/10 dilution (red) or a rabbit IgG negative control antibody (green).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53R2 antibody [EPR8816] (ab154194)

Immunohistochemical analysis of paraffin-embedded Human ovarian carcinoma tissue labeling p53R2 with unpurified ab154194 at 1/50 dilution.

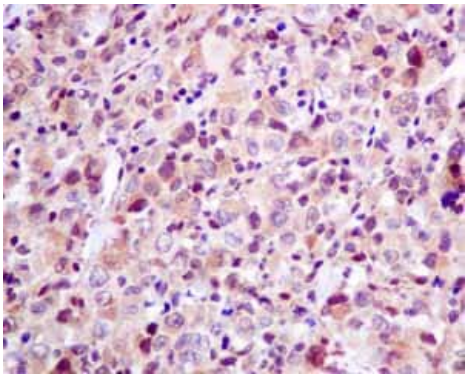
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53R2 antibody [EPR8816] (ab154194)

Immunohistochemical analysis of paraffin embedded Human urinary bladder transitional carcinoma tissue using unpurified ab154194 showing +ve staining.

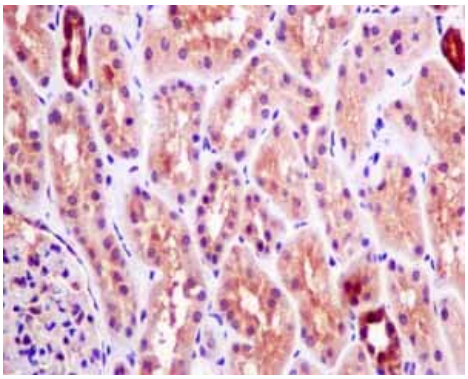
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53R2 antibody [EPR8816] (ab154194)

Immunohistochemical analysis of paraffin embedded Human melanoma tissue using unpurified ab154194 showing +ve staining.

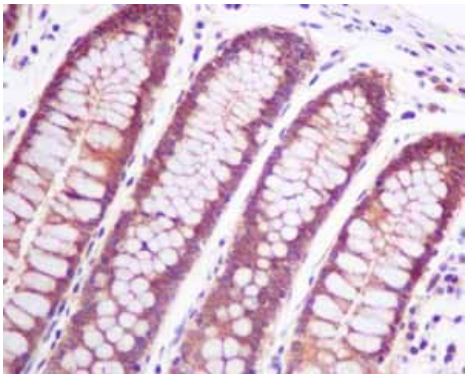
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53R2 antibody [EPR8816] (ab154194)

Immunohistochemical analysis of paraffin embedded Human normal kidney tissue unpurified ab154194 showing +ve staining.

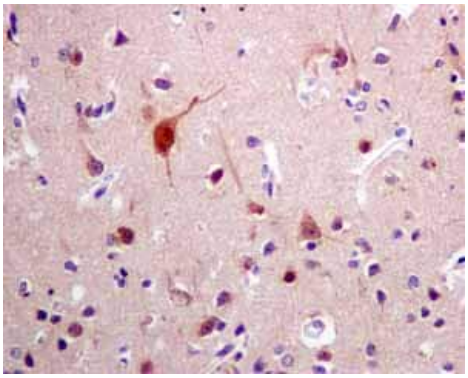
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53R2 antibody [EPR8816] (ab154194)

Immunohistochemical analysis of paraffin embedded Human normal colon tissue using unpurified ab154194 showing +ve staining.

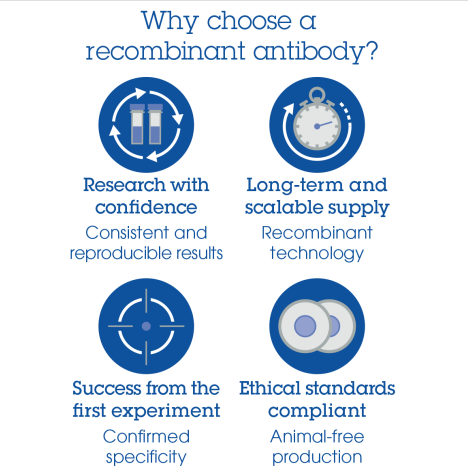
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p53R2 antibody [EPR8816] (ab154194)

Immunohistochemical analysis of paraffin embedded Human normal brain tissue using unpurified ab154194 showing +ve staining.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Anti-p53R2 antibody [EPR8816] (ab154194)

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