abcam

Product datasheet

Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade ab2769

9 Abreviews 44 References 11 Images

Overview

Product name	Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade
Description	Mouse monoclonal [RPT9] to Aryl hydrocarbon Receptor - ChIP Grade
Host species	Mouse
Specificity	This antibody specifically immunoprecipitates a single ~95 kDa protein representing AHR from Hepa 1 cytosol. Immunohistochemical staining of AHR in rat liver results in strong cytoplasmic and some nuclear staining.
Tested applications	**Suitable for:** ICC/IF, ELISA, WB, IHC-Fr, ChIP, IHC-P, IP, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Rabbit
Immunogen	Recombinant fragment corresponding to Mouse Aryl hydrocarbon Receptor aa 12-31. Sequence: R(12)KRRKP(17) V(22)KPIPAEGIK(31) Run BLAST with Run BLAST with
Positive control	rat liver tissue sections Hepa 1 cytosolic lysate

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.05% Sodium azide
Purity	Protein A purified
Clonality	Monoclonal
Clone number	RPT9
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab2769 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
ICC/IF	★★★★☆ (1)	1/10 - 1/100.
ELISA		Use at an assay dependent concentration.
WB	★★★☆☆ (2)	1/500 - 1/2000. PubMed: 21266776 Albeit some customers have been successful using Ab2769 for western blots with endogenous samples, we recommend using **ab190797** for this application.
IHC-Fr		Use at an assay dependent concentration. PubMed: 23036591
ChIP	★★★★☆ (2)	1/100.
IHC-P	★★★★★ (2)	Use at an assay dependent concentration.
IP	★★★★☆ (1)	Use at an assay dependent concentration.
Flow Cyt	★☆☆☆☆ (1)	Use 1-5µg for 10^6 cells. **ab18392** - Mouse monoclonal IgG3, is suitable for use as an isotype control with this antibody.

Target

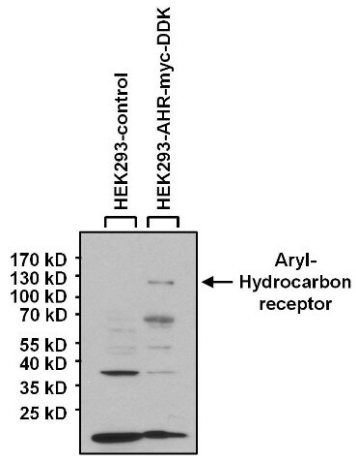
Function Ligand-activated transcriptional activator. Binds to the XRE promoter region of genes it activates. Activates the expression of multiple phase I and II xenobiotic chemical metabolizing enzyme genes (such as the CYP1A1 gene). Mediates biochemical and toxic effects of halogenated aromatic hydrocarbons. Involved in cell-cycle regulation. Likely to play an important role in the development and maturation of many tissues.

Tissue specificity Expressed in all tissues tested including blood, brain, heart, kidney, liver, lung, pancreas and skeletal muscle.

Sequence similarities Contains 1 basic helix-loop-helix (bHLH) domain.
Contains 1 PAC (PAS-associated C-terminal) domain.
Contains 2 PAS (PER-ARNT-SIM) domains.

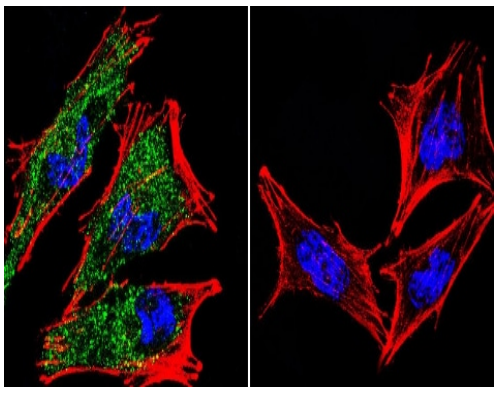
Cellular localization Cytoplasm. Nucleus. Initially cytoplasmic; upon binding with ligand and interaction with a HSP90, it translocates to the nucleus.

Images



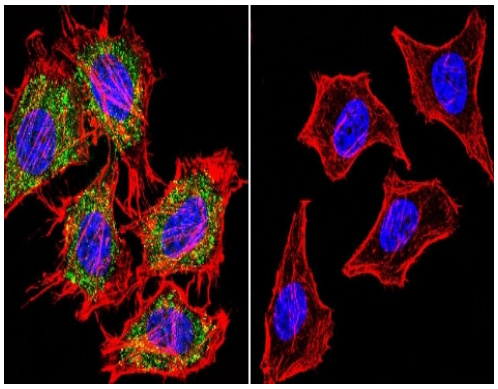
Western blot - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

Western blot analysis of Aryl Hydrocarbon Receptor was performed by loading 40 ug of HEK293 lysate overexpressing Aryl Hydrocarbon Receptor (right lane) or empty vector control (left lane) and 10ul of a Prestained Protein Ladder onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with an Aryl Hydrocarbon Receptor monoclonal antibody (ab2769) at a dilution of 1:1000 overnight at 4°C on a rocking platform then washed in TBS-0.1%Tween-20 and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:20,000 for 1 hour. Chemiluminescent detection was performed.



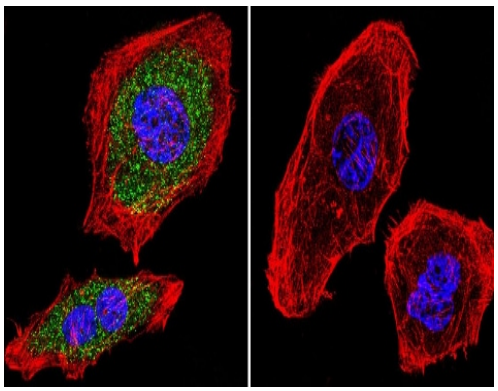
Immunocytochemistry/ Immunofluorescence - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

Immunocytochemistry/Immunofluorescence analysis of Aryl Hydrocarbon Receptor shows staining in A2058 cells. Aryl Hydrocarbon Receptor (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab2769 (1:20) overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.



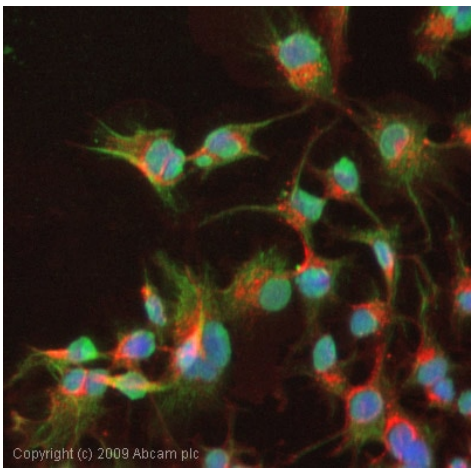
Immunocytochemistry/ Immunofluorescence - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

Immunocytochemistry/Immunofluorescence analysis of Aryl Hydrocarbon Receptor shows staining in HeLa cells. Aryl Hydrocarbon Receptor (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab2769 (1:20) overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.



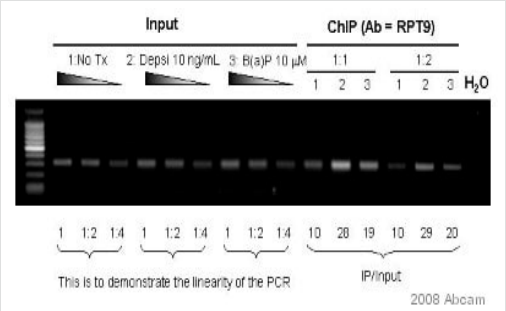
Immunocytochemistry/ Immunofluorescence - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

Immunocytochemistry/Immunofluorescence analysis of Aryl Hydrocarbon Receptor shows staining in U251 cells. Aryl Hydrocarbon Receptor (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were incubated without (control) or with ab2769 (1:20) overnight at 4°C, washed with PBS and incubated with a DyLight-488 conjugated secondary antibody. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

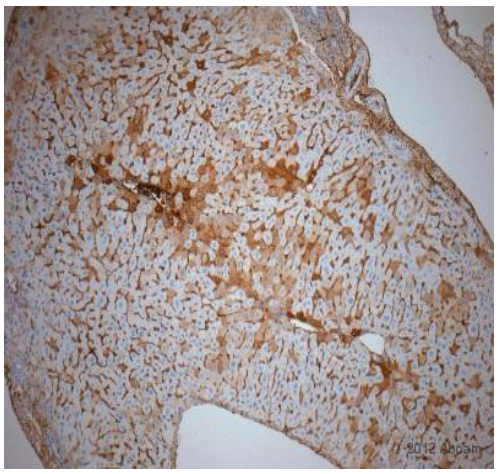
ICC/IF image of ab2769 stained HepG2 cells. The cells were methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab2769, in a 1/200 dilution) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



ChIP - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

Image courtesy of an anonymous Abreview.

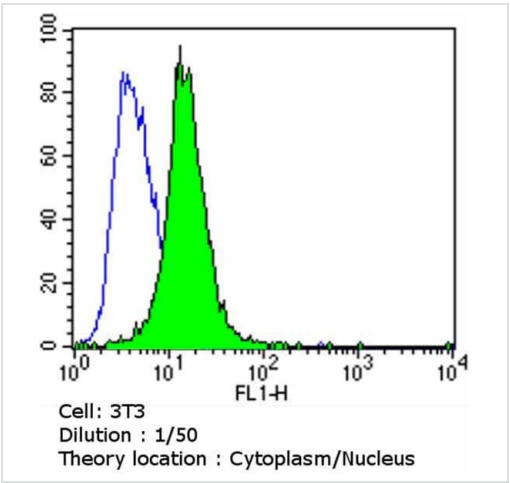
This was a trial experiment to evaluate the association of AhR to the ABCG2 promoter using ab2769 at a 1/100 dilution for the IP (ChIP assay). Semiquantitative PCR was performed to evaluate the relative association of AhR with the proximal ABCG2 promoter in a S1 colon cancer cell line without treatment, or treated with depsipeptide (10 ng/mL 24h) or benzo(a)pyrene (10uM 24h).
Cross-linking (X-ChIP) - 10 Mins 0 Secs
Lane 1: DNA ladder (100 bp from promega)
Inputs (lanes 2-4): S1 no treatment - serial dilution
Inputs (lanes 5-7): S1 treated with depsipeptide - serial dilution
Inputs (lanes 8-10): S1 treated with benzo(a)pyrene - serial dilution
AhR ChIP (lane 11-13): use 1:1 diluted immunoprecipitate for PCR
Lane 11=S1 no treatment lane 12=S1 depsipeptide 10ng/mL lane 13= S1 benzo(a)pyrene 10uM
AhR ChIP (lane 14-16): use 1:2 diluted immunoprecipitate for PCR same order as lanes 11-13
Lane 17: H2O control for PCR.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

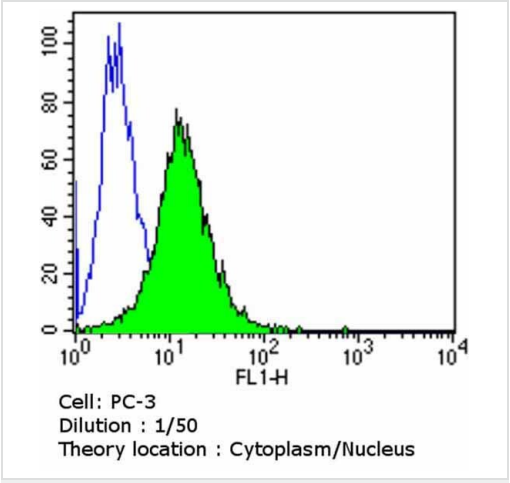
This image is courtesy of an anonymous Abreview

ab2769 staining Aryl hydrocarbon Receptor in Mouse liver tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/750) for 1 hour. Ab98784 (1/500) was used as the secondary antibody. Background staining due to secondary with positive stainind seen in the cytoplasm of the hepatocytes



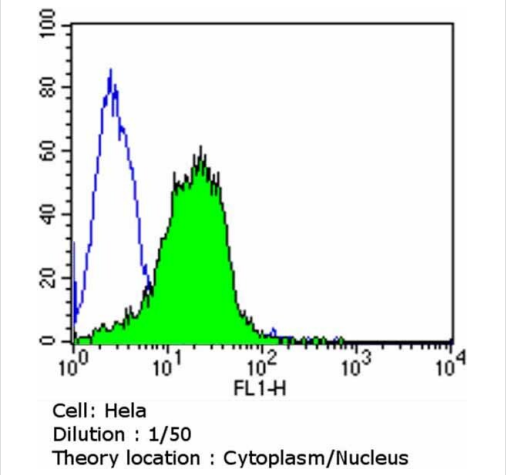
Flow Cytometry - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

Flow cytometry analysis of Aryl Hydrocarbon Receptor showing positive staining in the nucleus and cytoplasm of NIH/3T3 cells compared to an isotype control (blue). Cells were harvested and adjusted to a concentration of 1-5x10^6 cells/ml. Cells were then fixed with 2% paraformaldehyde and washed with PBS. Cells were penetrated by dropping the supernatant and adding 90% methanol followed by incubation for 10 minutes at room temperature. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2769 at 1:50 for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.



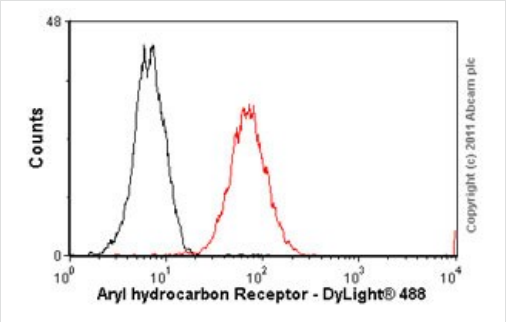
Flow Cytometry - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

Flow cytometry analysis of Aryl Hydrocarbon Receptor showing positive staining in the nucleus and cytoplasm of PC-3 cells compared to an isotype control (blue). Cells were harvested and adjusted to a concentration of 1-5x10^6 cells/ml. Cells were then fixed with 2% paraformaldehyde and washed with PBS. Cells were penetrated by dropping the supernatant and adding 90% methanol followed by incubation for 10 minutes at room temperature. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2769 at 1:50 for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.



Flow Cytometry - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

Flow cytometry analysis of Aryl Hydrocarbon Receptor showing positive staining in the nucleus and cytoplasm of Hela cells compared to an isotype control (blue). Cells were harvested and adjusted to a concentration of 1-5x10^6 cells/ml. Cells were then fixed with 2% paraformaldehyde and washed with PBS. Cells were penetrated by dropping the supernatant and adding 90% methanol followed by incubation for 10 minutes at room temperature. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2769 at 1:50 for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.



Flow Cytometry - Anti-Aryl hydrocarbon Receptor antibody [RPT9] - ChIP Grade (ab2769)

Overlay histogram showing HEK293 cells stained with ab2769 (red line). The cells were fixed with 100% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab2769, 1/20 dilution) for 30 min at 22°C. The secondary antibody used was a goat **anti mouse-DyLight® 488** (IgG H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HEK293 cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

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