abcam

Product datasheet

Anti-Oct4 antibody [EPR17980] ab200834

Recombinant RabMAb

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Overview

Product name	Anti-Oct4 antibody [EPR17980]
Description	Rabbit monoclonal [EPR17980] to Oct4
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra), ChIP-sequencing, ChIC/CUT&RUN-seq
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: NCCIT, Ntera-2/D1 and F9 whole cell lysates. IHC-P: Human dysgerminoma of ovary and spermatocytoma tissues. ICC/IF: NCCIT cells, F9 cells Flow Cyt (intra): F9 cells. IP: F9 whole cell lysate. ChIP-seq: NCCIT 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**.

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: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17980

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab200834 in the following tested applications.

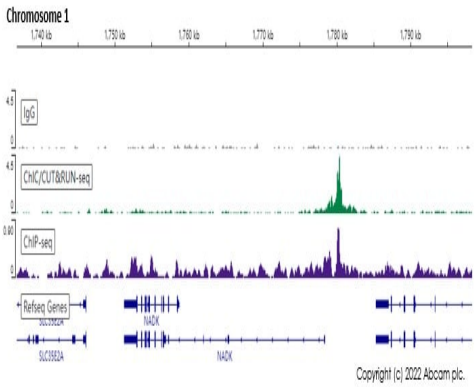
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/10000. Detects a band of approximately 45 kDa (predicted molecular weight: 38 kDa).
IHC-P	★★★★★ (1)	1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (1)	Use a concentration of 5 µg/ml.
IP		1/30.
Flow Cyt (Intra)		1/60. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP-sequencing		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg

Target

Function	Transcription factor that binds to the octamer motif (5'-ATTTGCAT-3'). Forms a trimeric complex with SOX2 on DNA and controls the expression of a number of genes involved in embryonic development such as YES1, FGF4, UTF1 and ZFP206. Critical for early embryogenesis and for embryonic stem cell pluripotency.
Tissue specificity	Expressed in developing brain. Highest levels found in specific cell layers of the cortex, the olfactory bulb, the hippocampus and the cerebellum. Low levels of expression in adult tissues.
Sequence similarities	Belongs to the POU transcription factor family. Class-5 subfamily. Contains 1 homeobox DNA-binding domain. Contains 1 POU-specific domain.
Developmental stage	Highly expressed in undifferentiated embryonic stem cells and expression decreases gradually after embryoid body (EB) formation.
Domain	The POU-specific domain mediates interaction with PKM2.
Post-translational modifications	Sumoylation enhances the protein stability, DNA binding and transactivation activity. Sumoylation is required for enhanced YES1 expression. Ubiquitinated; undergoes 'Lys-63'-linked polyubiquitination by WWP2 leading to proteasomal degradation.
Cellular localization	Nucleus. Expressed in a diffuse and slightly punctuate pattern.

Images

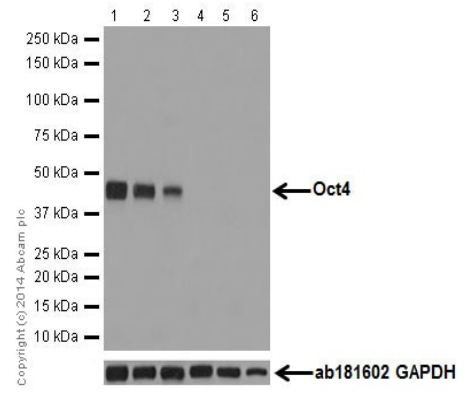


ChIC/CUT&RUN sequencing - Anti-Oct4 antibody [EPR17980] (ab200834)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/µL, 2 x 10^5 NCCIT (Human pluripotent embryonic carcinoma cell line) cells and 5 µg of ab200834 [EPR17980]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown. The ChIP data was conducted on chromatin prepared from NCCIT cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 NCCIT cells and 8 µg of ab200834. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-Oct4 antibody [EPR17980] (ab200834)

All lanes : Anti-Oct4 antibody [EPR17980] (ab200834) at 1/10000 dilution

Lane 1 : NCCIT (Human pluripotent embryonic carcinoma) whole cell lysate
Lane 2 : NTera-2/D1 (Human malignant pluripotent embryonic carcinoma) whole cell lysate
Lane 3 : F9 (Mouse embyro testicular cancer cell line) whole cell lysate
Lane 4 : JAR (Human placenta choriocarcinoma cell line) whole cell lysate
Lane 5 : RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate
Lane 6 : Mouse testis lysate

Lysates/proteins at 10 µg per lane.

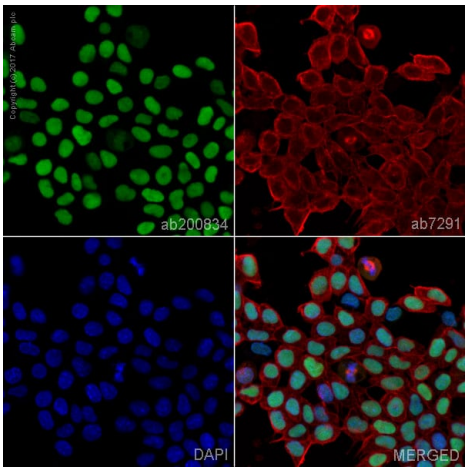
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 38 kDa
Observed band size: 45 kDa

Exposure time: 30 seconds

Low levels of expression in adult tissues.

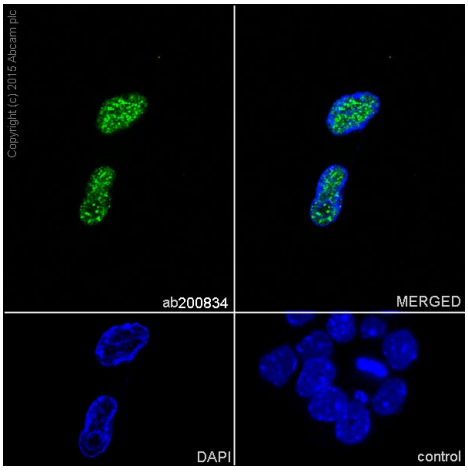
Blocking/Dilution Buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Oct4 antibody [EPR17980] (ab200834)

ab200834 staining Oct4 in F9 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab200834 at a 5µg/ml concentration and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1µg/ml concentration, followed by a further incubation at room temperature for 1h with an anti-rabbit AlexaFluor® 488 (**ab150081**) at 2 µg/ml (shown in green) and an anti-mouse AlexaFluor® 594 (**ab150120**) at 2 µg/ml (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).

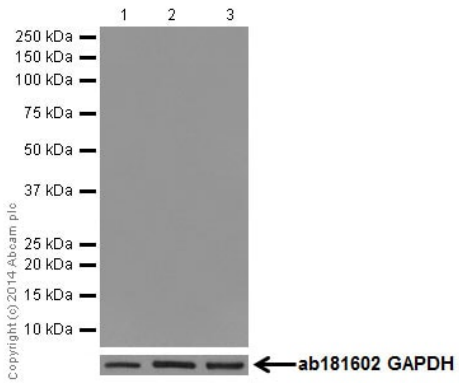
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-Oct4 antibody [EPR17980] (ab200834)

Immunocytochemistry/Immunofluorescence analysis of F9 (mouse embryonal carcinoma) labelling Oct4 with purified ab200834 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Control: PBS only



Western blot - Anti-Oct4 antibody [EPR17980] (ab200834)

All lanes : Anti-Oct4 antibody [EPR17980] (ab200834) at 1/1000 dilution

Lane 1 : Human placenta lysate

Lane 2 : Human fetal brain lysate

Lane 3 : Human testis lysate

Lysates/proteins at 10 µg per lane.

Secondary

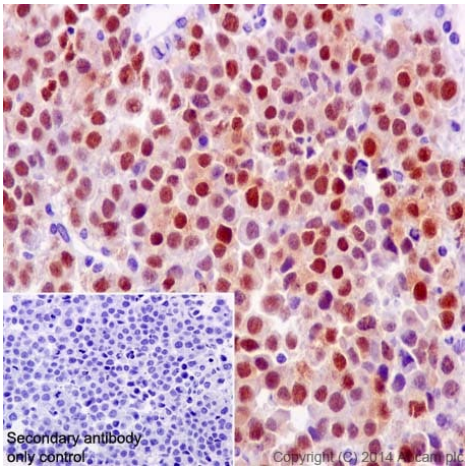
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 38 kDa

Exposure time: 3 minutes

Low levels of expression in adult tissues

Blocking/Dilution Buffer: 5% NFDM/TBST.

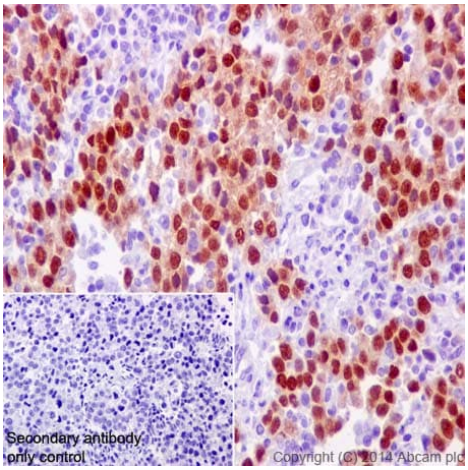


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Oct4 antibody [EPR17980] (ab200834)

Immunohistochemical analysis of paraffin-embedded Human dysgerminoma of ovary tissue labeling Oct4 with ab200834 at 1/500 dilution, followed Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear and weakly cytoplasmic staining on Human dysgerminoma of ovary tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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

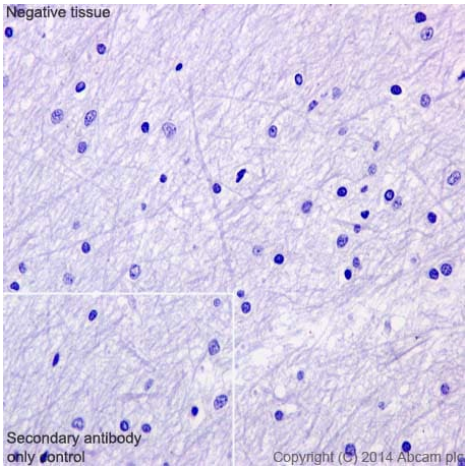


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Oct4 antibody [EPR17980] (ab200834)

Immunohistochemical analysis of paraffin-embedded Human spermatocytoma tissue labeling Oct4 with ab200834 at 1/500 dilution, followed Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear and weakly cytoplasmic staining on Human spermatocytoma tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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

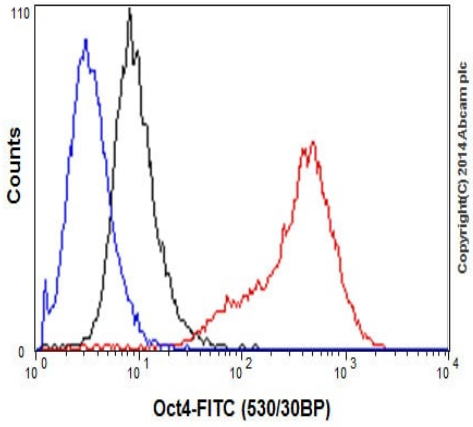


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Oct4 antibody [EPR17980] (ab200834)

Immunohistochemical analysis of paraffin-embedded Human cerebral cortex tissue labeling Oct4 with ab200834 at 1/500 dilution, followed Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Human cerebral cortex tissue is a negative control for Oct4. Counter stained with Hematoxylin.

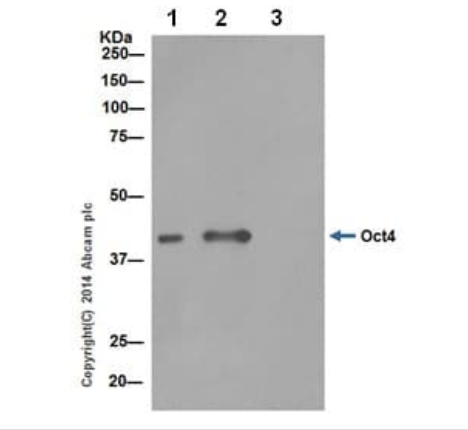
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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



Flow Cytometry (Intracellular) - Anti-Oct4 antibody [EPR17980] (ab200834)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed F9 (Mouse embyro testicular cancer cell line) cells labeling Oct4 with ab200834 at 1/60 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 1/150 dilution was used as the secondary antibody.

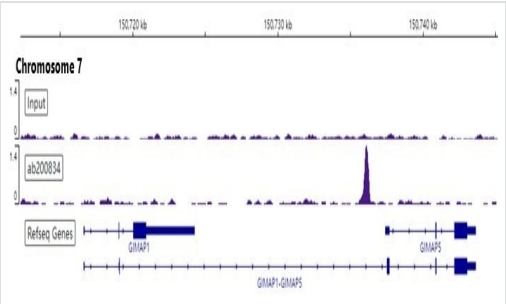


Immunoprecipitation - Anti-Oct4 antibody [EPR17980] (ab200834)

Oct4 was immunoprecipitated from 1mg of F9 (Mouse embyro testicular cancer cell line) whole cell lysate with ab200834 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab200834 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: F9 whole cell lysate 10 µg (Input). Lane 2: ab200834 IP in F9 whole cell lysate. Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab200834 in F9 whole cell lysate.
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

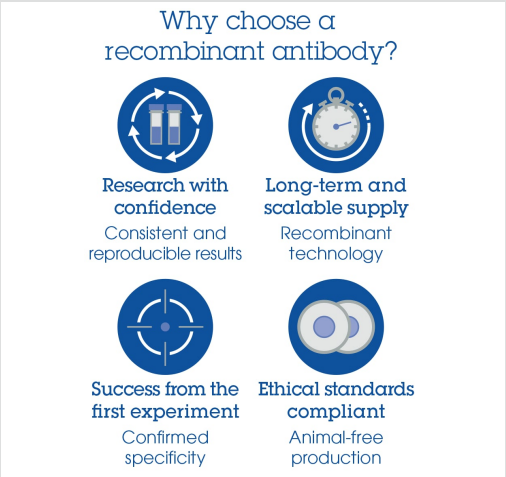
Exposure time: 5 seconds



ChIP-sequencing - Anti-Oct4 antibody [EPR17980] (ab200834)

Chromatin was prepared from NCCIT (Human pluripotent embryonic carcinoma cell line) cells. ChIP was performed with 10^7 NCCIT cells and 8 µg of ab200834 [EPR17980]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded **here**.



Anti-Oct4 antibody [EPR17980] (ab200834)

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