abcam

Product datasheet

Anti-Oct4 antibody ab19857

44 Abreviews 525 References 7 Images

Overview

Product name	Anti-Oct4 antibody
Description	Rabbit polyclonal to Oct4
Host species	Rabbit
Specificity	Previous batches of ab19857 have worked well in IHC, but recent batches of this antibody are showing non-specific staining on day 6 mouse testis in our lab (as of Nov 2016). Replenishment batches of our polyclonal antibody, ab19857 are tested in WB. Previous batches were additionally validated in ICC/IF and IP. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative recombinant antibody, **ab181557**.
Tested applications	**Suitable for:** IP, ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Sheep, Rhesus monkey
Immunogen	Synthetic peptide corresponding to Human Oct4 aa 300 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as **ab20650**)
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: 99% PBS

	Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help
Purity	Protein A purified
Purification notes	Affinity Purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab19857 in the following tested applications.

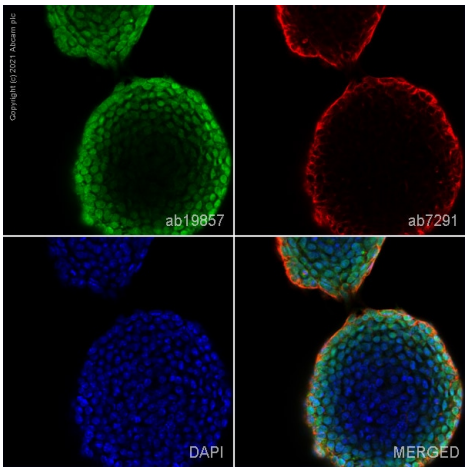
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
ICC/IF	★★★★★ (11)	Use a concentration of 1 - 5 µg/ml.
WB	★★★★☆ (10)	Use a concentration of 1 µg/ml. Detects a band of approximately 43 kDa (predicted molecular weight: 39 kDa).

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

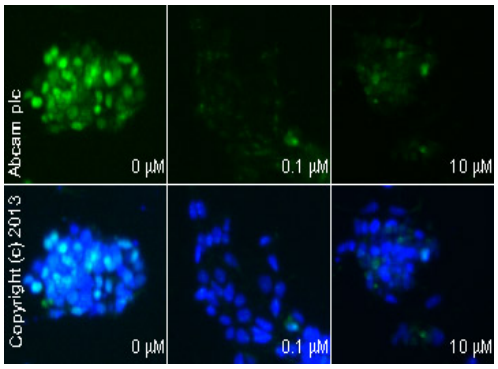


Immunocytochemistry/ Immunofluorescence - Anti-Oct4 antibody (ab19857)

ab19857 staining Oct4 in mES cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween 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 ab19857 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

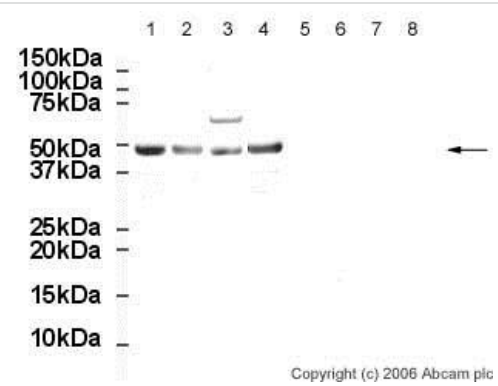
Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



Immunocytochemistry/ Immunofluorescence - Anti-Oct4 antibody (ab19857)

ab19857 staining Oct4 in F9 cells treated with trans-retinoic acid (**ab120728**) The cells were incubated at 37°C for 2 days in media containing different concentrations of **ab120728** (trans-retinoic acid) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab19857 (1 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (**ab96899**) at 1:250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Western blot - Anti-Oct4 antibody (ab19857)

All lanes : Anti-Oct4 antibody (ab19857) at 1 µg/ml

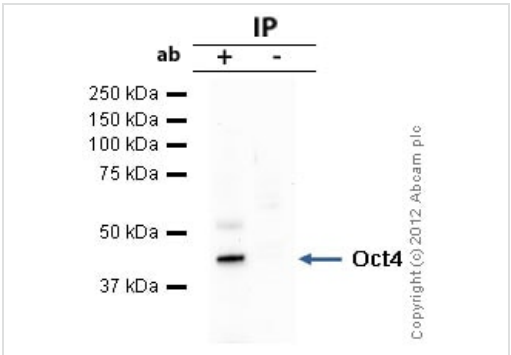
Lane 1 : MEL-1 (Human embryonic stem cell, male cell line) Whole Cell Lysate (**ab27198**)
Lane 2 : MEL-2 (Human embryonic stem cell, female cell line) Whole Cell Lysate (**ab27196**)
Lane 3 : IOUD2 (Mouse embryonic stem cell, selected for Oct4 expression cell line) Whole Cell Lysate (**ab27202**)
Lane 4 : F9 (Mouse embryonic carcinoma cell line) Whole Cell Lysate (**ab27193**)
Lane 5 : MEL-1 (Human embryonic stem cell, male cell line) Whole Cell Lysate (**ab27198**) with Human Oct4 peptide (**ab20650**) at 1 µg/ml
Lane 6 : MEL-2 (Human embryonic stem cell, female cell line) Whole Cell Lysate (**ab27196**) with Human Oct4 peptide (**ab20650**)

at 1 µg/ml
Lane 7 : lOUD2 (Mouse embryonic stem cell, selected for Oct4 expression cell line) Whole Cell Lysate (**ab27202**) with Human Oct4 peptide (**ab20650**) at 1 µg/ml
Lane 8 : F9 (Mouse embryonic carcinoma cell line) Whole Cell Lysate (**ab27193**) with Human Oct4 peptide (**ab20650**) at 1 µg/ml

Lysates/proteins at 10 µl per lane.

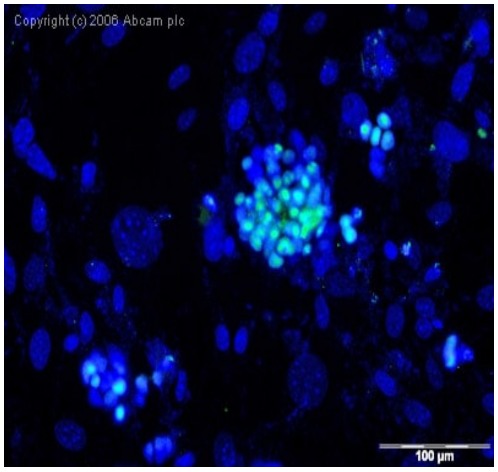
Predicted band size: 39 kDa
Observed band size: 48 kDa
Additional bands at: 65 kDa. We are unsure as to the identity of these extra bands.

MEL-1 and MEL-2 Human Embryonic Stem Cell Lysates (**ab27198** and **ab27196**), lOUD2 Oct4-expressing Mouse Embryonic Stem Cell Lysate (**ab27202**) and F9 Mouse Embryonic Carcinoma Cell Lysate (**ab27193**) all contain Oct4 protein. As expected, a band corresponding to Oct4 was detected in all four of the lysates by Westen Blot using anti-Oct4 antibody ab19857. Each of the Oct4 bands was specifically blocked using the immunising peptide of ab19857. The origin of the additional 65 kDa band detected in Lane 3 is unknown and may be a non-specific band that is recognised, in addition to Oct4, by ab19857 in mouse embryonic stem cells.



Immunoprecipitation - Anti-Oct4 antibody (ab19857)

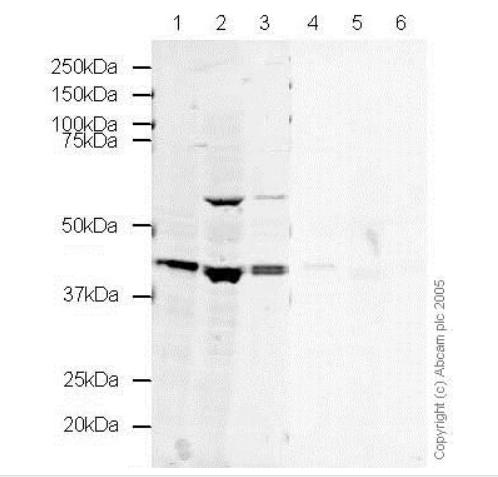
Oct4 was immunoprecipitated using 0.5mg E14Tg2a whole cell extract, 5µg of Rabbit polyclonal to Oct4 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, E14Tg2a whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab19857.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).
Band: 48kDa: Oct4.



Immunocytochemistry/ Immunofluorescence - Anti-Oct4 antibody (ab19857)

ICC/IF image of ab19857 stained Mouse Embryonic Stem cells. The cells were PFA fixed (4% PFA, 20 min) and incubated with the antibody (ab19857, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT™ FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue).

The large nuclei of the Feeder cells can be seen in the image; ab19857 does not localise to these nuclei. ab19857 can be seen localising to the much smaller nuclei of the Mouse Embryonic Stem cells.



Western blot - Anti-Oct4 antibody (ab19857)

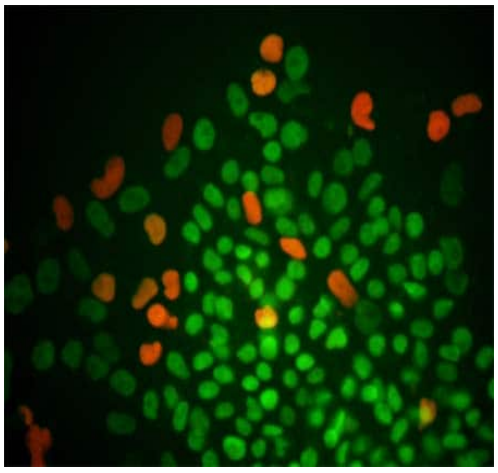
All lanes : Anti-Oct4 antibody (ab19857) at 1 µg/ml

Lane 1 : Human embryonic stem cell lysate
Lane 2 : Mouse embryonic stem cell lysate
Lane 3 : Mouse embryonic germ cell lysate
Lane 4 : Human embryonic stem cell lysate with Human Oct4 peptide (**ab20650**)
Lane 5 : Mouse embryonic stem cell lysate with Human Oct4 peptide (**ab20650**)
Lane 6 : Mouse embryonic germ cell lysate with Human Oct4 peptide (**ab20650**)

Lysates/proteins at 20 µg per lane.

Predicted band size: 39 kDa
Observed band size: 43 kDa
Additional bands at: 55 kDa (possible glycosylated form), 55 kDa (possible cross reactivity)

Anti-Oct4 antibody ab19857 only detected a band corresponding to the expected size of Oct4 in human ES cell lysate. In mouse ES and EG cell lysates, ab19857 detected a band of approximately 55 kDa in addition to the expected 39 kDa Oct4 band.



Immunocytochemistry/ Immunofluorescence - Anti-Oct4 antibody (ab19857)

This image is courtesy of Ludovic Vallier, University of Cambridge

The image shows a colony of differentiating Human Embryonic Stem Cells double-stained with anti-Oct4 antibody ab19857 (green) and Sox17 antibody (red). The nuclei of Oct-4-positive undifferentiated hESCs stained bright green. Staining was restricted to the nuclei. Oct4-positive nuclei were Sox17-negative, and vice versa. This antibody can be used as a marker of Oct4-positive undifferentiated Human Embryonic Stem Cells.

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