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Datasheet for ABIN968417

anti-OCT4 antibody (AA 252-372)

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Overview

Quantity:	50 µg
Target:	OCT4 (POU5F1)
Binding Specificity:	AA 252-372
Reactivity:	Human, Mouse
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This OCT4 antibody is un-conjugated
Application:	Western Blotting (WB), Immunofluorescence (IF), Biolmaging (BI)

Product Details

Immunogen:	Mouse Oct3 aa. 252-372 Recombinant Protein
Clone:	40-Oct
Isotype:	IgG1 kappa
Cross-Reactivity:	Human
Characteristics:	<ol style="list-style-type: none">1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.4. Please refer to us for technical protocols.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity

Product Details

chromatography.

Target Details

Target: OCT4 (POU5F1)

Alternative Name: Oct-3 ([POU5F1 Products](#))

Background: Development of a multicellular organism from a single fertilized cell is regulated by the coordinated activity of DNA transcription factors. Oct3/4, a member of the POU family of transcription factors, functions in pluripotent cells of early embryonic stem cell (ES) lines and embryonal carcinomas (EC). Other members of the POU family include Oct1, Oct2, Pit-1, and unc-86. The POU domain, a 150-amino acid region that determines binding specificity, is conserved among these proteins and consists of 3 subdomains: POU-specific A and B subdomains and a homeobox-like subdomain. Oct3/4 is expressed in undifferentiated cells, but is lost as cells are induced to differentiate. Oct3/4 is not expressed in adult tissues. The interaction of Oct3/4 with SOX2, another embryonic transcription factor, produces an active complex that regulates expression of genes such as Nanog, UTF1, and FGF4. Although Oct3/4 is specifically phosphorylated on serine residues, this modification is not required for DNA binding, but may affect its transactivation potential. Thus, Oct3/4 is a transcription factor that plays an important role in determining early steps of embryogenesis and differentiation.

Synonyms: Oct3, OTF3, Oct4, OTF4, POU5F1

Molecular Weight: 46 kDa

Pathways: [Stem Cell Maintenance](#)

Application Details

Application Notes: Bioimaging

1. Seed the cells in appropriate culture medium at an appropriate cell density in an 96-well Imaging Plate , and culture overnight to 48 hours.
2. Remove the culture medium from the wells, wash the wells twice with 100 µl of 1x PBS, and fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or fixation buffer to each well and incubating for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and wash the wells twice with 100 µl of 1x PBS.
4. Permeabilize the cells by adding 100 µl of 1x Perm/Wash™ buffer to each well and incubating for 30 minutes at RT.
5. Remove the permeabilizer, and wash the wells twice with 100 µl of 1x PBS.

Application Details

6. Dilute the antibody in Perm/Wash™ buffer, and stain the cells by adding 50 µl of the diluted antibody to each well and incubating for 1 hour at RT.
7. Remove the diluted antibody, and wash the wells three times with 100 µl of 1x PBS.
8. Remove the PBS, dilute the second-step reagent in Perm/Wash™ buffer, and stain the cells by adding 50 µl of the diluted second-step reagent to each well and incubating for 1 hour at RT.
9. Remove the diluted second-step reagent, and wash the wells twice with 100 µl of 1x PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 in 1x PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Comment: Related Products: ABIN968631, ABIN967389

Restrictions: For Research Use only

Handling

Format: Liquid

Concentration: 250 µg/mL

Buffer: Aqueous buffered solution containing BSA, glycerol, and ≤0.09 % sodium azide.

Preservative: Sodium azide

Precaution of Use: This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

Storage: -20 °C

Storage Comment: Store undiluted at -20° C.

Publications

Product cited in: Pan, Thomson: "Nanog and transcriptional networks in embryonic stem cell pluripotency." in: **Cell research**, Vol. 17, Issue 1, pp. 42-9, (2007) ([PubMed](#)).

Nishimoto, Fukushima, Okuda, Muramatsu: "The gene for the embryonic stem cell coactivator UTF1 carries a regulatory element which selectively interacts with a complex composed of Oct-3/4 and Sox-2." in: **Molecular and cellular biology**, Vol. 19, Issue 8, pp. 5453-65, (1999) ([PubMed](#)).

Vigano, Staudt: "Transcriptional activation by Oct-3: evidence for a specific role of the POU-

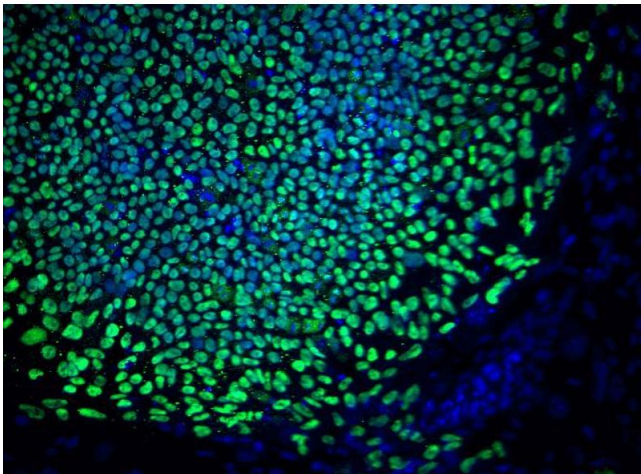
specific domain in mediating functional interaction with Oct-1." in: **Nucleic acids research**, Vol. 24, Issue 11, pp. 2112-8, (1996) ([PubMed](#)).

Yuan, Corbi, Basilico, Dailey: "Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3." in: **Genes & development**, Vol. 9, Issue 21, pp. 2635-45, (1995) ([PubMed](#)).

Rosfjord, Scholtz, Lewis, Rizzino: "Phosphorylation and DNA binding of the octamer binding transcription factor Oct-3." in: **Biochemical and biophysical research communications**, Vol. 212, Issue 3, pp. 847-53, (1995) ([PubMed](#)).

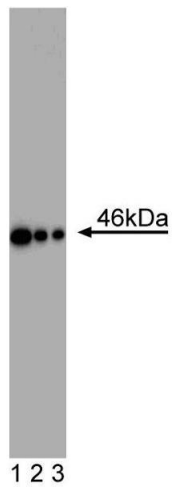
There are more publications referencing this product on: [Product page](#)

Images



Immunofluorescence

Image 1. Bottom Row: Immunofluorescent staining of human and mouse ES cell lines. The H9 cell line (First Panel) and ES-E14TG2a cells (Second Panel) were cultured, fixed, permeabilized, and stained with Purified Oct3/4 monoclonal antibody (pseudo-colored green) according to the Recommended Assay Procedure. The second-step reagent was Alexa Fluor® 555 goat anti-mouse Ig (Invitrogen) and counter-staining was with Hoechst 33342 (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer using a 10X objective (H9) or 20X objective (E14) and merged using BD Attovision™ software. Permeabilization using BD Perm/Wash(TM) was used with this antibody, Triton™ X-100 or cold methanol will also work.



Western Blotting

Image 2. Western Blot analysis of Oct3/4 in human and mouse ES cell lines. Lysates from H9 human ES cells (WiCell, Madison, WI, left blot) and ES-E14TG2a mouse ES cells (ATCC CRL-1821, right blot) and were probed with Purified Mouse anti-Oct3/4 monoclonal antibody at titrations of 2.0 (lanes 1), 1.0 (lanes 2), and 0.5 µg/ml (lanes 3). Oct3/4 is identified as a band of 46 kDa in the human and mouse ES cells.

The H9 cells were cultured on a mitomycin C-treated mouse embryonic fibroblast feeder layer [MEF (CF-1), ATCC SCRC-1040] that maintains the undifferentiated state of the ES cells. The lysate was made from a mixture of the 2 cell types, the majority of which were H9 cells.

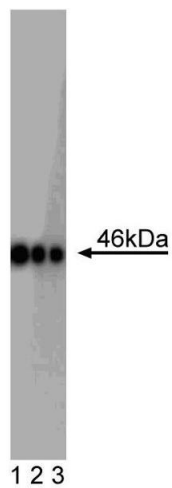


Image 3. ES-E14TG2a mouse ES cells (ATCC CRL-1821)