





OCT4 Monoclonal Antibody (9B7)

Catalog Number MA1-104 Product data sheet

Details	
Size	100 µg
Host/Isotope	Mouse / IgG1
Class	Monoclonal
Туре	Antibody
Clone	9B7
Immunogen	Full length human recombinant protein of human POU5F1 produced in E.coli.
Conjugate	Unconjugated
Form	Liquid
Concentration	1 mg/mL
Purification	Protein A
Storage buffer	PBS with 1mg/mL BSA, 30% glycerol
Contains	0.05% sodium azide
Storage Conditions	-20°C

Species Reactivity	
Species reactivity	Human, Mouse
Published species	Human
rubilstieu species	Tullian
Tested Applications	Dilution *
Flow Cytometry (Flow)	3-5 μg/1x10^6 cells
Western Blot (WB)	1:500-1:5,000
Immunocytochemistry (ICC/IF)	1-2 μg/mL
Published Applications	
Immunocytochemistry (ICC/IF)	See 6 publications below
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Flow Cytometry (Flow)	See 3 publications below

^{*} Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

MA1-104 detects Oct4/Pou5F1 in Human embryonic carcinomic samples, but not in adult samples (e.g. HeLa cells). This antibody did not show reactivity in a mouse embryonic cell line (i.e. F9 embryonic carcinoma cells). MA1-104 has been successfully used in FACS, IF, and WB. By Western blot, this antibody detects a band at 40 kDa in human NCCIT and NTERA-2 embryonal carcinoma cells, but not in negative control cells.

Background/Target Information

POU5F1, also commonly known as Oct-4, is a maternally expressed octamer-binding protein that was the first transcription factor described for the early stages of development. The role of POU5F1 in embryonic development suggested that it might be useful in the creation of stem cells that might be useful in cell replacement therapies in the treatment of several degenerative diseases. Artificial stem cells, termed induced pluripotent stem (iPS) cells, can be created by expressing POU5F1 and the transcription factors Sox2, Klf4 and Lin28 along with c-Myc in mouse fibroblasts. More recently, experiments have demonstrated that iPS cells could be generated using expression plasmids expressing POU5F1, Sox2, KlfF4 and c-Myc, eliminating the need for virus introduction, thereby addressing a safety concern for potential use of iPS cells in regenerative medicine.

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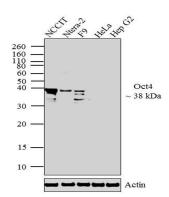
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Product Images For OCT4 Monoclonal Antibody (9B7)



OCT4 Antibody (MA1-104)

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell models owing to their inherent genetic constitution. Expression of Oct4 was observed specifically in embryonic carcinoma cell lines such as NCCIT, NTERA2 and F9 using Oct4 Mouse Monoclonal Antibody (Product # MA1-104) in western blot. {RE}



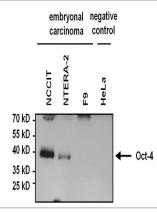
Oct4

~ 38 kDa

Actin

OCT4 Antibody (MA1-104) in WB

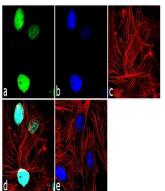
Western blot analysis was performed on whole cell extracts (30 µg lysate) of NCCIT (Lane1), Ntera-2 (Lane2), F9 (Lane 3), HeLa (Lane 4) and Hep G2 (lane 5). The blots were probed with Anti-Oct-4 Mouse Monoclonal Antibody (Product # MA1-104, 1:500-1:2000 µg/mL) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP conjugate (Product # 62-6520, 1:4000 dilution). A 38 kDa corresponding to Oct-4 was observed across cell lines tested, expect in HeLa and Hep G2. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 12 % Bis-Tris gel (Product # NP0342BOX), XCell SureLock™ Electrophoresis System (Product # El0002), and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody using iBind™ Flex Western Starter Kit (Product # SLF2000S). Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



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OCT4 Antibody (MA1-104) in WB

Western blot analysis of Oct4 was performed by loading 75 μ g of the indicated whole cell lysates and 5 μ L of the Lane Marker Reducing Sample Buffer (Product # 39000) per well onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% BSA/TBST (Product # 37525) for at least 1 hour. The membrane was probed with an Oct4 monoclonal antibody (Product # MA1-104) at a dilution of 1:1000 overnight at 4° C on a rocking platform, washed in TBS-0.1%Tween 20, and probed with a goat anti-mouse IgG-HRP secondary antibody (Product # 32430) at a dilution of 1:20,000 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Dura (Product # 34075). **Note: the antibody is detecting Oct4 in the human embryonal carcinoma cells, but not in negative control HeLa cells. Unknown bands at 80 kDa were also observed with this antibody.



OCT4 Antibody (MA1-104) in ICC/IF

Immunofluorescence analysis of Oct4/Pou5F1 was done on 70% confluent log phase NTREA-2 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with Oct4/Pou5F1 (9B7) Mouse Monoclonal Antibody (Product # MA1-104) at 2 µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor® 555 Rhodamine Phalloidin (Product # R415, 1:300). Panel d is a merged image showing nuclear localization. Panel e is a no primary antibody control. The images were captured at 60X magnification.

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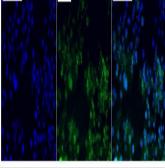
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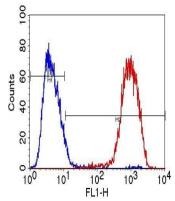
OCT4 Antibody (MA1-104) in ICC/IF

Immunofluorescent analysis of Oct4 (green) in retinoic acid-treated NCCIT cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 0.3% BSA/TBST (Product # 37525) for 15 minutes at room temperature. Cells were probed with an Oct4 monoclonal antibody (Product # MA1-104) at a dilution of 1:100 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Thermo Scientific ArrayScan at 20X magnification. **Note: the absence of Oct4 staining in a subset of the NCCIT cells suggests differentiation. In addition to the nuclear staining of Oct4, the antibody also shows some cytoplasmic staining.



OCT4 Antibody (MA1-104) in Flow

Flow cytometry analysis of Oct4 (red histogram) on human NCCIT cells. Cells were harvested, fixed with 4% formaldehyde, washed with PBS, and incubated with an Oct4 monoclonal antibody (Product # MA1-104) at a 1:100 dilution or PBS alone (blue histogram) for 1 hour on ice. For flow analysis, a 30-minute incubation with DyLight 488 goat anti-mouse IgG secondary antibody (Product # 35502) was performed and 10,000 cells were acquired for each





6 Immunocytochemistry	y References
Species / Dilution	Summary
	MA1-104 was used in Immunocytochemistry to establish two iPSC lines from a patient harbouring a rare homozygous splice site variant in GLP1R for use as a model to study the molecular pathology of GLP1R dysfunction.
Human / 1:100	Stem cell research (2020; 50:) "Generation of iPSC lines (KAUSTi011-A, KAUSTi011-B) from a Saudi patient with epileptic encephalopathy carrying homozygous mutation in the GLP1R gene." Author(s):Alowaysi M,Astro V,Fiacco E,Alzahrani F,Alkuraya FS,Adamo A PubMed Article URL:http://dx.doi.org/10.1016/j.scr.2020.102148
	MA1-104 was used in Flow Cytometry to describe the derivation of multiple 47-XXY iPSC lines from three unrelated KS patients to study the impact of supernumerary X chromosome during early development.
Human / 1:50	Stem cell research (2020; 49:) "Establishment of an iPSC cohort from three unrelated 47-XXY Klinefelter Syndrome patients (KAUSTi007-A, KAUSTi007-B, KAUSTi009-A, KAUSTi009-B, KAUSTi010-A, KAUSTi010-B)." Author(s):Alowaysi M,Fiacco E,Astro V,Adamo A PubMed Article URL:http://dx.doi.org/10.1016/j.scr.2020.102042
	MA1-104 was used in Immunocytochemistry to derived iPSC lines from a high-grade 49-XXXXY Klinefelter Syndrome and two healthy donors' fibroblasts.
Human / 1:100	Stem cell research (2020; 49:) "Establishment of iPSC lines from a high-grade Klinefelter Syndrome patient (49-XXXXY) and two genetically matched healthy relatives (KAUSTi003-A, KAUSTi004-A, KAUSTi004-B, KAUSTi005-A, KAUSTi005-B, KAUSTi005-C)." Author(s):Alowaysi M,Fiacco E,Astro V,Adamo A PubMed Article URL:http://dx.doi.org/10.1016/j.scr.2020.102008
Human / 1:100	MA1-104 was used in Immunocytochemistry to generate two iPSC lines 48-XXXY and 49-XXXXY from a non-mosaic 49-XXXXY KS patient carrying a balanced translocation t(4,11) (q35,q23).
	Stem cell research (2020; 49:) "Generation of two iPSC lines (KAUSTi001-A, KAUSTi002-A) from a rare high-grade Klinefelter Syndrome patient (49-XXXXY) carrying a balanced translocation t(4,11) (q35,q23)." Author(s):Alowaysi M,Fiacco E,Astro V,Adamo A PubMed Article URL:http://dx.doi.org/10.1016/j.scr.2020.102098
	MA1-104 was used in Immunocytochemistry-immunoflourescence to show that all tested coatings were highly comparable to the animal-derived Matrigel for both hiPSC maintenance and differentiation into renal podocyte-like cells.
Human / 1:500	ALTEX (2023; 40: 141) "Comparison of human recombinant protein coatings and fibroblast-ECM to Matrigel for induced pluripotent stem cell culture and renal podocyte differentiation." Author(s):Murphy C,Naderlinger E,Mater A,Kluin RJC,Wilmes A PubMed Article URL:http://dx.doi.org/10.14573/altex.2112204
	MA1-104 was used in Immunocytochemistry to show efficient generation of iPSCs from the elderly may provide a source of cells for the regeneration of tissues and organs with autologous cells as well as cellular models for the study of aging, longevity and age-related diseases.
Human / Not Cited	PloS one (2020; 14:) "Applying hydrodynamic pressure to efficiently generate induced pluripotent stem cells via reprogramming of centenarian skin fibroblasts." Author(s):Vosough M,Ravaioli F,Zabulica M,Capri M,Garagnani P,Franceschi C,Piccand J,Kraus MR,Kannisto K,Gramignoli R,Strom SC PubMed Article URL:http://dx.doi.org/10.1371/journal.pone.0215490
3 Flow Cytometry Refer	rences
Species / Dilution	Summary
	MA1-104 was used in Flow Cytometry to describe the derivation of multiple 47-XXY iPSC lines from three unrelated KS patients to study the impact of supernumerary X chromosome during early development.
Human / 1:50	Stem cell research (2020; 49:) "Establishment of an iPSC cohort from three unrelated 47-XXY Klinefelter Syndrome patients (KAUSTi007-A, KAUSTi007-B, KAUSTi009-B, KAUSTi010-A, KAUSTi010-B)." Author(s):Alowaysi M,Fiacco E,Astro V,Adamo A

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Human / 1:200	MA1-104 was used in Flow cytometry/Cell sorting to show that using naturally isogenic iPSCs from mosaic Klinefelter Syndrome patients to compare disease and healthy cells carrying a virtually identical genomic background.
	Stem cell research (2020; 49:) "Derivation of two naturally isogenic iPSC lines (KAUSTi006-A and KAUSTi006-B) from a mosaic Klinefelter Syndrome patient (47-XXY/46-XY)." Author(s):Fiacco E,Alowaysi M,Astro V,Adamo A PubMed Article URL:http://dx.doi.org/10.1016/j.scr.2020.102049
Human / Not Cited	MA1-104 was used in Flow cytometry/Cell sorting to prove the existence of a divergent scaffolding role of KDM1A splice variants, independent of their enzymatic activity, during hESC differentiation into cardiac cells.
	iScience (2022; 25:) "Fine-tuned KDM1A alternative splicing regulates human cardiomyogenesis through an enzymatic-independent mechanism." Author(s):Astro V,Ramirez-Calderon G,Pennucci R,Caroli J,Saera-Vila A,Cardona-Londoño K,Forastieri C,Fiacco E, Maksoud F,Alowaysi M,Sogne E,Falqui A,Gonzàlez F,Montserrat N,Battaglioli E,Mattevi A,Adamo A PubMed Article URL:http://dx.doi.org/10.1016/j.isci.2022.104665

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