

# Product Datasheet

## SIX2 Antibody (3D7) - Azide and BSA Free H00010736-M01

Unit Size: 0.1 mg

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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**H00010736-M01**

SIX2 Antibody (3D7) - Azide and BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	Concentrations vary lot to lot. See vial label for concentration. If unlisted please contact technical services.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	3D7
Preservative	No Preservative
Isotype	IgG1 Kappa
Purity	IgG purified
Buffer	In 1x PBS, pH 7.4

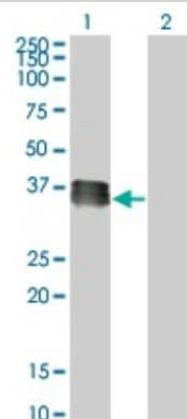
Product Description	
Description	Novus Biologicals Mouse SIX2 Antibody (3D7) - Azide and BSA Free (H00010736-M01) is a monoclonal antibody validated for use in IHC, WB, ELISA, Flow and ICC/IF. Anti-SIX2 Antibody: Cited in 12 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	10736
Gene Symbol	SIX2
Species	Human, Mouse, Rat
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 23996934). Rat reactivity reported in scientific literature (PMID: 26295710).
Specificity/Sensitivity	SIX2 - sine oculis homeobox homolog 2 (Drosophila)
Immunogen	SIX2 (AAH24033, 1 a.a. ~ 291 a.a) full-length recombinant protein with GST tag. MW of the GST tag alone is 26 KDa. MSMLPTFGFTQEQVACVCEVLQQGGNIERLGRFLWSPACEHLHKNESVLKA KAVVAFHRGNFRELKILESHQFSPHNHAKLQQLWLKAHYIEAEKLRGRPLGA VGKYRVRKFPPLPRSIWDGEETSFCFKEKRSVLREWYAHNPYPSPREKREL TEATGLTTTQVSNWFKNRRQRDRAAEAKERENNENSNSNSHNPPLNGSGKSVL GSSEDEKTPSGTPDHSSSSPALLLSPPPPGLPSLHSLGHPPGPSAVPVPVPGG GGADPLQHHHGLQDSILNPMSANLVDLGS
Notes	This product is produced by and distributed for Abnova, a company based in Taiwan.

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, ELISA, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Knockdown Validated
Recommended Dilutions	Western Blot 1:100-1:2000, Flow Cytometry, ELISA 1:100-1:2000, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry-Paraffin, Knockdown Validated
Application Notes	Antibody reactive against transfected lysate and recombinant protein for western blot. It has also been used for ELISA and RNAi Validation. Use in FLOW reported in scientific literature (PMID: 26295710). Use in Immunocytochemistry/immunofluorescence reported in scientific literature (PMID: 23996934).

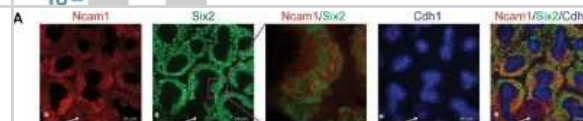


## Images

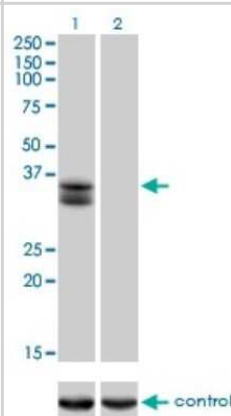
Western Blot: SIX2 Antibody (3D7) [H00010736-M01] - Analysis of SIX2 expression in transfected 293T cell line by SIX2 monoclonal antibody (M01), clone 3D7. Lane 1: SIX2 transfected lysate (32.3 kDa). Lane 2: Non-transfected lysate.



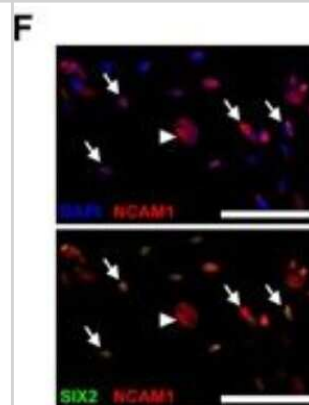
Immunocytochemistry/Immunofluorescence: SIX2 Antibody (3D7) [H00010736-M01] - NCAM1 expression in mouse embryonic kidney organ and hFK serum-free cultures. Mouse embryonic kidney organ culture stained for Ncam1, Six2 and E-cad as indicated. An enlargement of the Ncam/Six2 signal is shown to emphasize the nuclear localization of Six2. White arrow illustrates the absence of Six2 signal in E-cad positive cells. An occurrence of Six2/E-cad positive cells is indicated with the asterisk. Images were obtained using Nikon A1R confocal microscope with and processed in ImageJ/Fiji software. Image collected and cropped by CiteAb from the following publication (<https://embomolmed.embopress.org/cgi/doi/10.1002/emmm.201201584>), licensed under a CC-BY license.



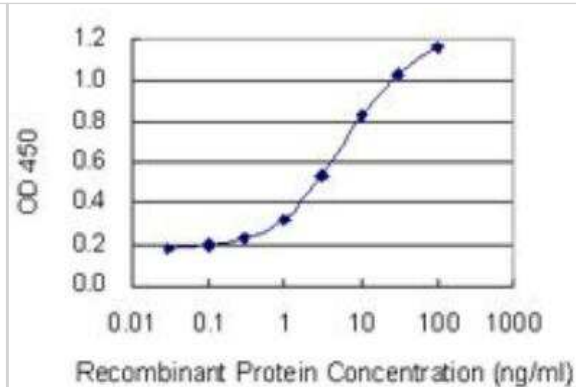
Western Blot: SIX2 Antibody (3D7) [H00010736-M01] - Analysis of SIX2 over-expressed 293 cell line, cotransfected with SIX2 Validated Chimera RNAi (Cat # H00010736-R01V) (Lane 2) or non-transfected control (Lane 1). Blot probed with SIX2 monoclonal antibody (M01), clone 3D7 (Cat # H00010736-M01). GAPDH (36.1 kDa) used as specificity and loading control.



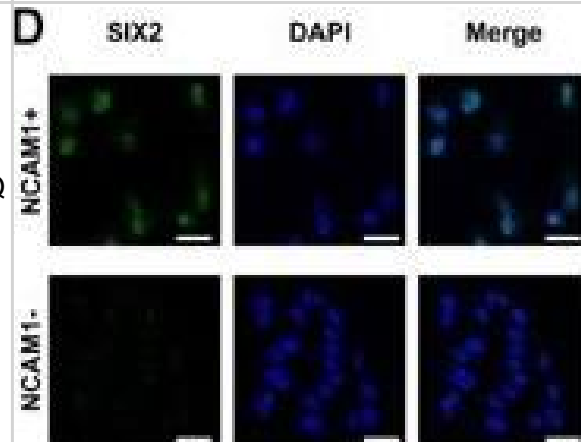
Immunocytochemistry/Immunofluorescence: SIX2 Antibody (3D7) [H00010736-M01] - Characterization of immunosorted NCAM1+ subpopulation. Double labelling of sorted NCAM1+ cells for NCAM1 and SIX2: NCAM1 with DAPI (upper panel; red and blue channels), NCAM1 with SIX2 (lower panel; red and green channels), indicating both NCAM1+ SIX2+ (arrows) and NCAM1+ SIX2- (arrowheads) cells. Image collected and cropped by CiteAb from the following publication (<https://embomolmed.embopress.org/cgi/doi/10.1002/emmm.201201584>), licensed under a CC-BY license.



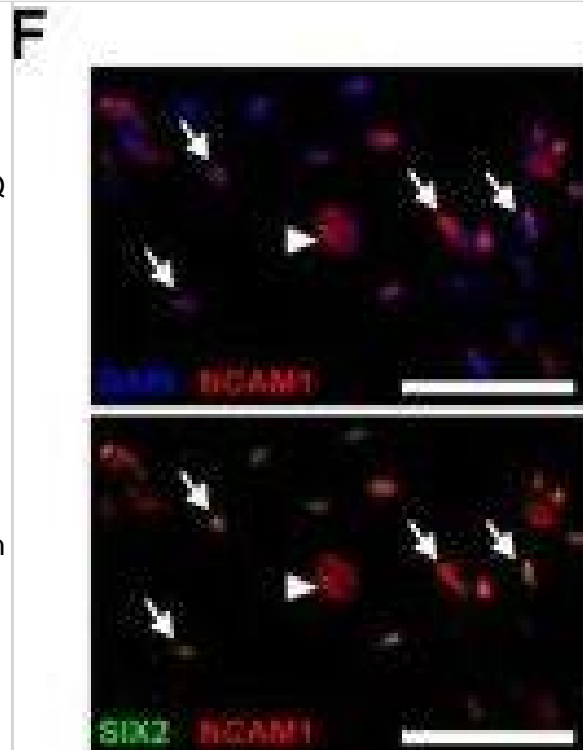
ELISA: SIX2 Antibody (3D7) [H00010736-M01] - Detection limit for recombinant GST tagged SIX2 is 0.03 ng/ml as a capture antibody.



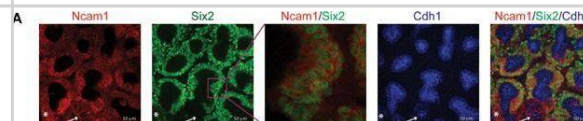
Immunocytochemistry/ Immunofluorescence: SIX2 Antibody (3D7) [H00010736-M01] - Characterization of immunosorted NCAM1+ subpopulation A. RT-PCR analysis of gene expression in NCAM1 cell fractions. GAPDH was used as endogenous control & NCAM1- cell were used as the calibrator sample for RQ calculation (therefore = 1). Data were analysed using SDS 3.2 software & presented as average RQ  $\pm$  SDEV of three replicates. \*\*\* $p < 0.001$ , \* $p < 0.05$  versus NCAM1-. B-D. Immunofluorescence staining of NCAM1+ & NCAM1- subpopulations for E-cadherin (E-cad) (B, red) vimentin (C, green) & SIX2 (D, green). Nuclei stained with Dapi (blue). (B-C) Images were obtained using Olympus DP72 camera attached to Olympus BX51 fluorescence microscope & processed via cellSens standard software, bar represents 200  $\mu$ m. (D) Images were obtained using Zeiss LSM 510 confocal microscope, bar represents 50  $\mu$ m. E. Fluorescent quantification of SIX2 immunostaining as represented in (D). F. Double labelling of sorted NCAM1+ cells for NCAM1 & SIX2: NCAM1 with DAPI (upper panel; red & blue channels), NCAM1 with SIX2 (lower panel; red & green channels), indicating both NCAM1+ SIX2+ (arrows) & NCAM1+ SIX2- (arrowheads) cells. G. Graph represents percentage of NCAM1+ SIX2+ cells & NCAM1+ SIX2- cells. H. Clonogenic efficiency of NCAM1+ cells sorted from hFK & cultured in SFM. Data are presented as average CE (%)  $\pm$  SDEV. \*\* $p < 0.01$  versus NCAM1-. I. Representative morphology of NCAM1+ & NCAM1- clones. Cells were observed using a Nikon Digital Sight camera attached to a Nikon Eclipse TS100 microscope. J. Clonogenic capacity of hFK cells treated with IMGN901 (ADC 55 nM), huN901 (Ab55 nM) or not treated (control). Data are presented as average CE (%)  $\pm$  SDEV. \*\*\* $p < 0.001$ , \* $p < 0.05$  versus control group. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/23996934>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: SIX2 Antibody (3D7) [H00010736-M01] - Characterization of immunosorted NCAM1+ subpopulationA. RT-PCR analysis of gene expression in NCAM1 cell fractions. GAPDH was used as endogenous control & NCAM1- cell were used as the calibrator sample for RQ calculation (therefore = 1). Data were analysed using SDS 3.2 software & presented as average RQ  $\pm$  SDEV of three replicates. \*\*\*p < 0.001, \*p < 0.05 versus NCAM1-.B-D. Immunofluorescence staining of NCAM1+ & NCAM1- subpopulations for E-cadherin (E-cad) (B, red) vimentin (C, green) & SIX2 (D, green). Nuclei stained with Dapi (blue). (B-C) Images were obtained using Olympus DP72 camera attached to Olympus BX51 fluorescence microscope & processed via cellSens standard software, bar represents 200  $\mu$ m. (D) Images were obtained using Zeiss LSM 510 confocal microscope, bar represents 50  $\mu$ m.E. Fluorescent quantification of SIX2 immunostaining as represented in (D).F. Double labelling of sorted NCAM1+ cells for NCAM1 & SIX2: NCAM1 with DAPI (upper panel; red & blue channels), NCAM1 with SIX2 (lower panel; red & green channels), indicating both NCAM1+ SIX2+ (arrows) & NCAM1+ SIX2- (arrowheads) cells.G. Graph represents percentage of NCAM1+ SIX2+ cells & NCAM1+ SIX2- cells.H. Clonogenic efficiency of NCAM1+ cells sorted from hFK & cultured in SFM. Data are presented as average CE (%)  $\pm$  SDEV. \*\*p < 0.01 versus NCAM1-.I. Representative morphology of NCAM1+ & NCAM1- clones. Cells were observed using a Nikon Digital Sight camera attached to a Nikon Eclipse TS100 microscope.J. Clonogenic capacity of hFK cells treated with IMG901(ADC 55 nM), huN901 (Ab55 nM) or not treated (control). Data are presented as average CE(%)  $\pm$  SDEV. \*\*\*p < 0.001, \*p < 0.05 versus control group. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/23996934>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: SIX2 Antibody (3D7) [H00010736-M01] - NCAM1 expression in mouse embryonic kidney organ & hFK serum-free culturesMouse embryonic kidney organ culture stained for Ncam1, Six2 & E-cad as indicated. An enlargement of the Ncam/Six2 signal is shown to emphasize the nuclear localization of Six2. White arrow illustrates the absence of Six2 signal in E-cad positive cells. An occurrence of Six2/E-cad positive cells is indicated with the asterisk. Images were obtained using Nikon A1R confocal microscope with & processed in ImageJ/Fiji software.Morphology of hFK cells cultured in SFM or SCM after 3 days (passage 0 Day 3—left panels) & towards confluence (14 days in SFM or 7 days in SCM—right panels). Distinct borders appear in SFM cultures (arrows) whereas cells with different morphology (arrows) are observed in SCM culture. Cells were observed using a Nikon Digital Sight camera attached to a Nikon Eclipse TS100 microscope.qRT-PCR analysis of gene expression in hFK cells cultured in SFM (three independent replicates). hPRT1 was used as endogenous control & SCM cells were used as the calibrator sample for RQ calculation (therefore = 1). Data were analysed using SDS 3.2 software.Representative FACS analysis of NCAM1 expression in hFK cells cultured in SFM at passage1. Data is presented in a histogram graph showing NCAM1 staining in blue & the isotype controls staining (negative control) in red.Immunofluorescence staining of NCAM1 (red) in total hFK cells cultured in SFM. Nuclei stained with Dapi (blue). Images were obtained using Olympus DP72 camera attached to Olympus BX51 fluorescence microscope & processed via cellSens standard software. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/23996934>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Huang B, Zeng Z, Li H et al. Modeling kidney development, disease, and plasticity with clonal expandable nephron progenitor cells and nephron organoids bioRxiv 2023-05-26 [PMID: 37293038]

Thao N, Bella R, Isabelle S et al. Functional differentiation and scalable production of renal proximal tubular epithelial cells from human pluripotent stem cells in a dynamic culture system. *Cell Prolif.* 2022-01-31 [PMID: 35102634]

Alexander M AP-2 $\beta$ /KCTD1 Control Distal Nephron Differentiation and Protect Against Renal Fibrosis. *Dev Cell.* 2020-06-16 [PMID: 32553120]

MS Rahman, W Wruck, LS Spitzhorn, L Nguyen, M Bohndorf, S Martins, F Asar, A Ncube, L Erichsen, N Graffmann, J Adjaye The FGF, TGF $\beta$  and WNT axis Modulate Self-renewal of Human SIX2+ Urine Derived Renal Progenitor Cells *Sci Rep*, 2020-01-20;10(1):739. 2020-01-20 [PMID: 31959818]

Reinke P and Kurtz A. Generating Multiple Kidney Progenitors and Cell Types from Human Pluripotent Stem Cells. *Methods Mol Biol.* 2019-01-01 [PMID: 30742266]

Monteiro Carvalho Mori da Cunha MG, Zia S, Oliveira Arcolino F et al. Amniotic Fluid Derived Stem Cells with a Renal Progenitor Phenotype Inhibit Interstitial Fibrosis in Renal Ischemia and Reperfusion Injury in Rats. *PLoS One* 2015-01-01 [PMID: 26295710] (FLOW, Rat)

Lee SH, Kim J, Ryu JY et al. Transcription coactivator Eya2 is a critical regulator of physiological hypertrophy. *J Mol Cell Cardiol.* 2011-12-14 [PMID: 22197309]

Murphy AJ, Pierce J, de Caestecker C et al. SIX2 and CITED1, markers of nephronic progenitor self-renewal, remain active in primitive elements of Wilms' tumor. *J Pediatr Surg.* 2012-06-01 [PMID: 22703800]

Wainwright EN, Wilhelm D, Combes AN et al. ROBO2 restricts the nephrogenic field and regulates Wolffian duct-nephrogenic cord separation. *Dev Biol.* 2015-06-23 [PMID: 26116176]

Mao Y, Francis-West P, Irvine KD. A Fat4-Dchs1 signal between stromal and cap mesenchyme cells influences nephrogenesis and ureteric bud branching. *Development.* 2015-06-26 [PMID: 26116666]

Hilliard Sylvia A, Yao Xiao, El-Dahr Samir S. Mdm2 is required for maintenance of the nephrogenic niche. *Dev Biol.* 2014-03-01 [PMID: 24440154] (Mouse)

Harari-Steinberg O, Metsuyanin S, Omer D et al. Identification of human nephron progenitors capable of generation of kidney structures and functional repair of chronic renal disease. *EMBO Mol Med.* 2013-09-02 [PMID: 23996934] (ICC/IF, Mouse)





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### **Products Related to H00010736-M01**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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