Technical Data Sheet

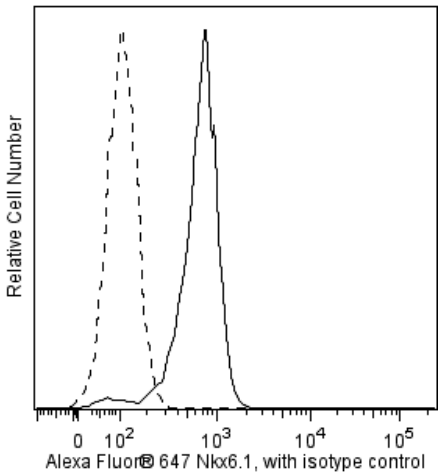
Alexa Fluor® 647 Mouse anti-Nkx6.1

Product Information

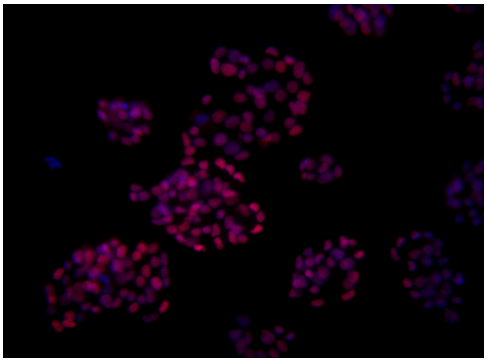
Material Number:	**563338**
Alternate Name:	Nkx6.1, NKX6-1, NKX6A, Nkx6-1, Nkx6.1, Nkx6a
Size:	50 tests
Vol. per Test:	5 µl
Clone:	R11-560
Immunogen:	Human Nkx6.1 Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Mouse Tested in Development: Human
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The R11-560 monoclonal antibody specifically binds to human and mouse homeobox protein Nkx6.1. The transcription factor Nkx6.1 plays a role in pancreatic islet beta cell development through the regulation of HNF1a and insulin genes. Nkx6.1 is considered to be a highly specific transcription factor marking pancreatic beta islet cells, where it is expressed in both developing and mature cells. In addition, Nkx6.1 functions as a transcriptional repressor induced by the Sonic Hedgehog (Shh) signaling pathway in fate determination of several neuronal cell types. During antibody development, the purified R11-560 monoclonal antibody was found to detect Nkx6.1 by western blot and indirect immunofluorescent staining followed by flow cytometric analysis or imaging analysis.



Flow cytometric analysis of Nkx6.1 expression in mouse pancreatic tumor (insulinoma) cells*. Beta-TC-6 (ATCC, CRL-11506™) cells were harvested with Accutase™ Cell Detachment Solution, fixed with BD Cytofix™ fixation buffer (Cat. No. 554655), and permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050). The cells were stained with either Alexa Fluor® 647 Mouse IgG1, κ isotype control (dashed line, Cat. No. 557714) or Alexa Fluor® 647 Mouse Anti-Nkx6.1 monoclonal antibody (solid line) at matched concentrations. Histograms were derived from gated events based on light scattering characteristics of Beta-TC-6 cells. Flow cytometry was performed on a BD LSRFortessa™ flow cytometry system. We do not recommend BD Phosflow™ Perm/Wash Buffer I for permeabilization.*



Immunofluorescent staining of Nkx6.1 in mouse pancreatic tumor (Insulinoma) cells. *Beta-TC-6 cells (ATCC, CRL-11506™) were fixed with BD Cytofix™ fixation buffer (Cat. No. 554655), permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050), and stained with Alexa Fluor® 647 Mouse anti-Human anti-Nkx6.1 (pseudo-colored red). Counter-staining was with DAPI (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer and merged using BD AttoVision™ Software.*

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Bioimaging	Tested During Development
Immunofluorescence	Tested During Development

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
557714	Alexa Fluor® 647 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21
561527	Accutase™ Cell Detachment Solution	100 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
4. 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.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
6. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. Accutase is a registered trademark of Innovative Cell Technologies, Inc.
10. All other brands are trademarks of their respective owners.
11. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
12. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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Inoue H, Rudnick A, German MS, Veile R, Donis-Keller H, Permutt MA. Isolation, characterization, and chromosomal mapping of the human Nkx6.1 gene (NKX6A), a new pancreatic islet homeobox gene. *Genomics.* 1997; 40:367-370. (Biology)

Nelson SB, Schaffer AE, Sander M. The transcription factors Nkx6.1 and Nkx6.2 possess equivalent activities in promoting beta-cell fate specification in Pdx1+ pancreatic progenitor cells. *Development.* 2007; 134(13):2491-2500. (Biology)

Pedersen IL, Klinck R, Hecksher-Sorensen J, et al. Generation and characterization of monoclonal antibodies against the transcription factor Nkx6.1. *J Histochem Cytochem.* 2006; 54(5):567-574. (Biology)

Prakash N, Puelles E, Freude K, et. al. Nkx6-1 controls the identity and fate of red nucleus and oculomotor neurons in the mouse midbrain. *Development.* 2009; 136(15):2545-2555. (Biology)

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