Technical Data Sheet

PerCP-Cy™5.5 Mouse anti-Human Pax-6

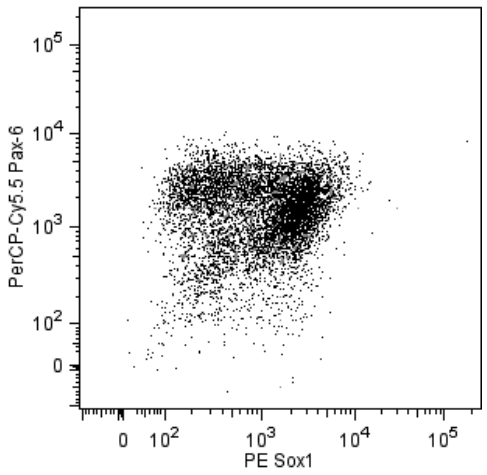
Product Information

Material Number:	**562388**
Alternate Name:	Oculorhombin, Aniridia type II protein, PAX6, AN2
Entrez Gene ID:	5080
Size:	50 tests
Vol. per Test:	5 µl
Clone:	O18-1330
Immunogen:	Human Pax-6 aa 406-422 Peptide
Isotype:	Mouse (BALB/c) IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Pax-6 is a member of the paired box (pax) gene family whose protein products are transcription factors involved in development. Pax family members share a highly conserved DNA binding domain that contains six alpha helices (paired domain) and a homeo box domain. Pax-6 has important roles in the development of the eye, nose, central nervous system, and pancreas. Defects in Pax-6 are responsible for various eye malformations including aniridia and Peters anomaly.

The O18-1330 monoclonal antibody reacts with human Pax-6. Because the Pax-6 protein sequence is highly conserved among vertebrate species, cross-reactivity with other species is possible.



Intracellular staining of Pax-6 in neural induction of human embryonic stem (ES) cells. *H9 human ES cells (WiCell, Madison, WI) were cultured in mTeSR® (Stem CellTechnologies) on plates coated with BD Matrigel™ hESC-qualified Matrix (Cat. No. 354277). Embryoid bodies (EB) were made and cultured in medium containing Knockout™ Serum Replacement (Life Technologies) without bFGF for 24 hours and then in medium containing 250 ng/ml human recombinant noggin (R&D Systems) and 10 mM SB 431542 (Tocris) for 4 more days. The EB were then plated on BD Matrigel-coated plates and grown in medium with ITS supplement (Sigma-Aldrich), noggin, and SB 431542. After growth for 7 days, the cells were collected, fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), and permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050). The cells were then stained with PerCP-Cy™5.5 Mouse anti-Human Pax-6 and PE Mouse anti-Human Sox1 (Cat. No.561592). The plot was derived from gated events based on light scattering characteristics for the neural induction. Flow cytometry was performed on a BD™ LSR II flow cytometry system. We do not recommend this conjugate for staining human ES-derived endoderm cells.*

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 with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Although this PerCP-Cy5.5 conjugate performs well when staining human ES-derived neural cells, we do not recommend it for staining human ES-derived endoderm cells. We recommend the PE (Cat. No. 561552) and Alexa Fluor® 488 (Cat. No. 561664) conjugates for staining human ES-derived endoderm cells.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Suggested Companion Products

Catalog Number	Name	Size	Clone
354277	BD Matrigel™ hESC-qualified Matrix, 5 ml vial	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
561592	PE Mouse anti-Human Sox1	50 tests	N23-844
554656	Stain Buffer (FBS)	500 ml	(none)
558020	PerCP-Cy™5.5 Mouse IgG2a, κ Isotype Control	50 tests	MOPC-173

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. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
4. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
5. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
10. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
11. All other brands are trademarks of their respective owners.
12. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Cerf ME. Transcription factors regulating beta-cell function. *Eur J Endocrinol.* 2006; 155(5):671-679. (Biology)

Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol.* 2009; 27(3):275-280. (Methodology)

Glaser T, Walton DS, Maas RL. Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. *Nat Genet.* 1992; 2:232-239. (Biology)

Osakada F, Jin ZB, Hirami Y, Ikeda H, Danjyo T, Watanabe K, Sasai Y, Takahashi M. In vitro differentiation of retinal cells from human pluripotent stem cells by small-molecule induction. *J Cell Sci.* 2009; 122:3169-3179. (Methodology)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**