

## Technical Data Sheet

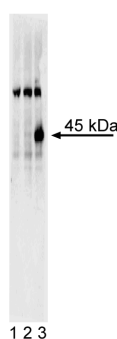
## Purified Mouse anti-Human Sox17

## Product Information

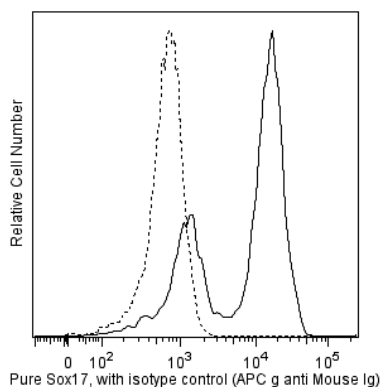
Material Number:	561590
Alternate Name:	SOX-17, SOX17, FLJ22252
Entrez Gene ID:	64321
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	P7-969
Immunogen:	Human Sox17 Recombinant Protein
Isotype:	Mouse (BALB/c) IgG1, $\kappa$
Reactivity:	QC Tested: Human
Target MW:	45 kDa
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

## Description

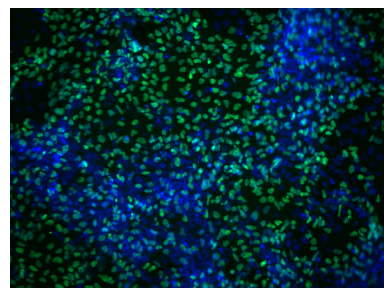
The P7-969 monoclonal antibody reacts with human Sox17, a member of the SOX (SRY-related HMG-box) family of transcription factors. SOX family members contain a DNA binding domain (HMG-box) and are involved in the control of development. Sox17 is expressed in primitive and definitive endoderm and regulates fetal and neonatal hematopoietic stem cell proliferation.



**Western blot analysis of Sox17 in definitive endoderm derived from human embryonic stem (ES) cells.** H9 human ES cells (WiCell, Madison, WI) were differentiated to definitive endoderm for 3 days (D'Amour et al, 2005) in RPMI medium supplemented with 0.5% FBS, 1 $\times$  L-glutamine, and 100 ng/ml Activin A (R&D Systems). Lysates from control ES cells (lane 1) and from day 1 (lane 2) and day 3 (lane 3) differentiated cells were probed with Purified Mouse anti-Human Sox17 antibody at 1.0  $\mu$ g/ml. The presence of Sox17 is demonstrated by the 45-kDa band in human ES-derived definitive endodermal cells (Lane 3), which is absent in H9 human ES cells (Lane 1) and at day 1 of differentiation (Lane 2). Purified Mouse anti-Hsp90 monoclonal antibody (Cat. No. 610418) was used as a gel-loading control (MW 90 kDa).



**Flow cytometric analysis of Sox17 in definitive endoderm derived from human embryonic stem (ES) cells.** H9 human ES cells (WiCell, Madison, WI) grown on an irradiated mouse embryonic feeder layer were differentiated to definitive endoderm for 3 days (D'Amour et al, 2005) in RPMI medium supplemented with 0.5% FBS, 1 $\times$  L-glutamine, and 100 ng/ml Activin A (R&D Systems). Day-3 differentiated cells were fixed with BD Cytotfix buffer (Cat. No. 554655) and permeabilized with BD™ Phosflow Perm buffer III (Cat. No. 558050). The cells were stained with either Purified Mouse IgG1,  $\kappa$  isotype control (dashed line, Cat. No. 555746) or Purified Mouse Anti-human Sox17 antibody (solid line) at matched concentrations. The second-step reagent was APC goat anti-mouse Ig (Cat. No. 550826). The histograms were derived from gated events based on light scattering characteristics of the H9-derived endoderm cells. Flow cytometry was performed on a BD LSR™ II flow cytometry system.



**Immunofluorescent staining of Sox17 in definitive endoderm derived from human embryonic stem (ES) cells.** H9 human ES cells (WiCell, Madison, WI) passage 35 grown on an irradiated mouse embryonic feeder layer were differentiated to definitive endoderm for 3 days (D'Amour et al, 2005) in RPMI medium supplemented with 0.5% FBS, 1 $\times$  L-glutamine, and 100 ng/ml Activin A (R&D Systems). The cells were fixed with BD Cytotfix buffer (Cat. No. 554655), permeabilized with 0.1% Triton™ X-100, and stained with Purified Mouse anti-Human Sox17 monoclonal antibody (pseudo-colored green) at 1.2  $\mu$ g/mL. The second-step reagent was Alexa Fluor® 488 goat anti-mouse Ig (Life Technologies), and counter staining was with Hoechst 33342 (pseudo-colored blue). The image was captured on a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ Software.

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## Preparation and Storage

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

Store undiluted at 4°C.

## Application Notes

### Application

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

## Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
555746	Purified Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554656	Stain Buffer (FBS)	500 ml	(none)

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.
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. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
6. Triton is a trademark of the Dow Chemical Company.

## References

- D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol.* 2005; 23(12):1534-1541. (Methodology: Cell differentiation)
- Katoh M. Molecular cloning and characterization of human SOX17. *Int J Mol Med.* 2002; 9(2):153-157. (Biology)
- Kim I, Saunders TL, Morrison SJ. Sox17 dependence distinguishes the transcriptional regulation of fetal from adult hematopoietic stem cells. *Cell.* 2007; 130(3):470-483. (Biology)
- Séguin CA, Draper JS, Nagy A, Rossant J. Establishment of endoderm progenitors by SOX transcription factor expression in human embryonic stem cells. *Cell Stem Cell.* 2008; 3(2):182-185. (Biology)
- Serrano AG, Gandillet A, Pearson S, Lacaud G, Kouskoff V. Contrasting effects of Sox17- and Sox18-sustained expression at the onset of blood specification. *Blood.* 2010; 115(19):3895-3898. (Biology)