

## Technical Data Sheet

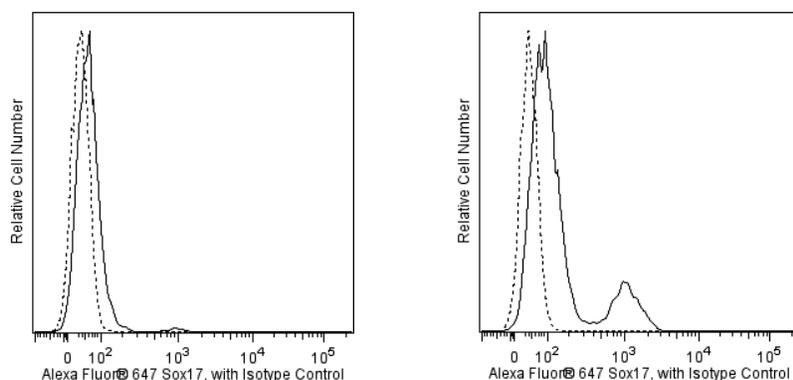
## Alexa Fluor® 647 Mouse Anti-Human Sox17

## Product Information

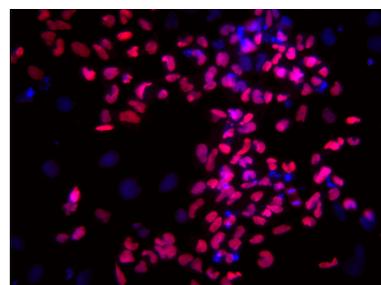
|                  |  |
|------------------|--|
| Material Number: | 562594   |
| Alternate Name:  | SOX-17, SOX17, FLJ22252  |
| Size:            | 50 tests   |
| Vol. per Test:   | 5 µl   |
| Clone:           | P7-969   |
| Immunogen:       | Human Sox17 Recombinant Protein  |
| Isotype:         | Mouse (BALB/c) IgG1, κ   |
| Reactivity:      | QC Testing: Human  |
| Storage Buffer:  | Aqueous buffered solution containing BSA, protein stabilizer, and ≤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.



**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, 1X L-glutamine, and 100 ng/ml Activin A (R&D Systems). Control ES cells (left panel) and day-3 differentiated cells (right panel) were 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 lines, Cat. No. 557714) or Alexa Fluor® 647 Mouse Anti-Human Sox17 antibody (solid lines) at matched concentrations. The histograms were derived from gated events based on light scattering characteristics of the human ES and H9-derived endoderm cells, respectively. Flow cytometry was performed on a BD™ LSRII flow cytometry system.



**Immunofluorescent staining of Sox17 on definitive endoderm derived from human embryonic stem (ES) cells.** H9 human ES cells (WiCell, Madison, WI) passage 46 grown on an irradiated mouse embryonic feeder layer were differentiated to definitive endoderm for 3 days in RPMI supplemented with 0.5% FBS, 1X L-glutamine, and 100 ng/ml Activin A (R&D Systems) (D'Amour et al, 2005). The cells were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), permeabilized with 0.1% Triton™-X 100, and stained with Alexa Fluor® 647 Mouse anti-Human Sox17 monoclonal antibody (pseudo-colored red) at 0.06 µg/mL. 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.

## 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          |
| Immunofluorescence                      | Tested During Development |

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## Suggested Companion Products

| Catalog Number | Name  | Size      | Clone   |
|----------------|---|-----------|---------|
| 554655         | Fixation Buffer                               | 100 ml    | (none)  |
| 558050         | Perm Buffer III                               | 125 ml    | (none)  |
| 557714         | Alexa Fluor® 647 Mouse IgG1 κ Isotype Control | 100 tests | MOPC-21 |

## Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100- $\mu$ l experimental sample (a test).
2. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.
3. 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.
4. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
7. An isotype control should be used at the same concentration as the antibody of interest.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. All other brands are trademarks of their respective owners.
10. Triton is a trademark of the Dow Chemical Company.
11. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

## 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)
- 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)

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