

Technical Data Sheet

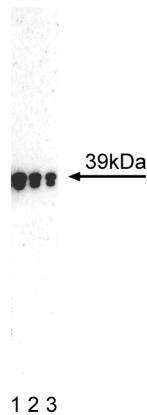
**Purified Mouse anti-Human SOX1**

**Product Information**

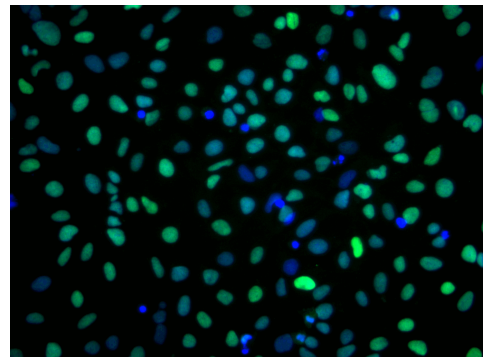
<b>Material Number:</b>	<b>560749</b>
<b>Size:</b>	0.1 mg
<b>Concentration:</b>	0.5 mg/ml
<b>Clone:</b>	N23-844
<b>Immunogen:</b>	Human Sox1 Peptide
<b>Isotype:</b>	Mouse IgG1, κ
<b>Reactivity:</b>	QC Testing: Human
<b>Target MW:</b>	39 kDa
<b>Storage Buffer:</b>	Aqueous buffered solution containing ≤0.09% sodium azide.

**Description**

The N23-844 monoclonal antibody reacts with human Sox1, a member of the SOX [SRY (sex determining region Y)-HMG-box] family of transcription factors. The encoded protein may act as a transcriptional activator after forming a protein complex with other proteins. It is one of the earliest transcription factors to be expressed in ectodermal cells committed to the neural fate. Sox1 is expressed in both embryonic and somatic neural stem and progenitor cells, and it is down regulated during neuronal differentiation in many neuronal subtypes.



**Western Blot analysis of SOX1.** Lysate from neural stem cells (NSCs) derived from H9 human ES cells (WiCell, Madison, WI) was probed with Purified Mouse anti-Human SOX1 monoclonal antibody at titrations of 0.5 (lane 1), 0.25 (lane 2), and 0.125 µg/ml (lane 3). SOX1 is identified as a tight doublet of almost 39 kDa.



**Immunofluorescent staining of Human SOX1.** NSCs derived from H9 human ES cells (WiCell Madison, WI) were fixed, permeabilized with BD Perm/Wash™ Buffer (Cat No. 554723), and stained with purified anti-human SOX1 (0.5 µg/test). 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 images were captured on a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ Software. Triton™ X-100 is also suitable for permeabilization.

**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**

Western blot	Routinely Tested
Bioimaging	Tested During Development
Flow cytometry	Tested During Development

**BD Biosciences**

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## Recommended Assay Procedure:

### Bioimaging

1. Seed the cells in appropriate culture medium at an appropriate cell density in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight to 48 hours.
2. Remove the culture medium from the wells, and wash (one to two times) with 100 µl of 1× PBS.
3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytotfix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 µl of 1× PBS.
5. Permeabilize the cells using either Triton™ X-100 (a) or Saponin (b):
  - a. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
  - b. Add 100 µl of 1× Perm/Wash buffer (Cat. No. 554723) to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps.
6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 µl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.b.).
7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT.
8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10.
9. Add 50 µl of diluted antibody per well and incubate for 60 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies.
10. Remove the antibody, and wash the wells three times with 100 µl of wash buffer. An optional detergent wash (100 µl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps.
11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody.
12. After the final wash, counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
13. View and analyze the cells on an appropriate imaging instrument.

### Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)

### Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to [wwwbdbiosciences.com/pharmingen/protocols](http://wwwbdbiosciences.com/pharmingen/protocols) for technical protocols.
3. Triton is a trademark of the Dow Chemical Company.
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
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.

### References

Kan L, Israsena N, Zhang Z, et al. Sox1 acts through multiple independent pathways to promote neurogenesis. *Dev Biol.* 2004; 15;26(2):580-594. (Biology)  
Malas S, Duthie SM, Mohri F, Lovell-Badge R, Episkopou V. Cloning and mapping of the human SOX1: a highly conserved gene expressed in the developing brain. *Mamm Genome.* 1997; 8(11):866-868. (Biology)  
Pevny LH, Sockanathan S, Placzek M, Lovell-Badge R. A role for SOX1 in neural determination. *Development.* 1998; 125(10):1967-1978. (Biology)  
Wilson M, Koopman P. Matching SOX: partner proteins and co-factors of the SOX family of transcriptional regulators. *Curr Opin Genet Dev.* 2002; 12(4):441-446. (Biology)

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