# Antibodies for stem cell research

## Using stem cell differentiation models to verify antibody specificity.

Due to their regenerative capabilities, stem cells have tremendous potential for use in cell therapy for degenerative disorders, including Alzheimer's and Parkinson's diseases, as well as for disease modeling, drug screening, and developmental biology research [1]. Stem cells are undifferentiated cells that have the capacity both to self-renew through mitosis and to differentiate into specialized cell types such as neuronal, liver, or muscle cells. Characterization of stem cells using antibodies is a critical step in stem cell research and relies on highly specific antibodies that perform well in the particular cell analysis platform.

### Use of stem cell differentiation models for antibody characterization

As vital reagents for stem cell research, antibodies that recognize specific stem cell biomarkers can be used for cell imaging, cell sorting, immunoassays, and other relevant applications. At the same time, stem cells differentiated along various lineages can be a powerful tool for determining the cell and tissue specificity of antibodies. In cases such as specialized neurons, where it is especially difficult to obtain the right cell model, neural stem cells differentiated into the required mature neurons can be a standardized way to validate neuron-specific antibodies. Here we highlight several examples of our use of stem cell differentiation models to evaluate antibody specificity.

### Antibodies for neuronal markers

Nestin and SOX2 are neuronal progenitor markers expressed on neuronal stem cells (NSCs). To confirm the specificity of our anti-nestin and anti-SOX2 antibodies, we tested them with human neural stem cells (NSCs) differentiated from H9-derived embryonic stem cells (ESCs) and observed the expected expression patterns (Figures 1A and 1B). Similarly, antibodies against mature neuronal markers MAP2 and β-III tubulin (Figures 1C and 1D) and GFAP (Figure 1E) were tested with differentiated neurons derived using Gibco™ StemPro™ NSC SFM (Neural Stem Cell Serum-Free Medium).

### ABfinity antibodies for developmental markers

We are also continuing to develop Invitrogen™ ABfinity™ recombinant antibodies for important developmental transcription factors. ABfinity antibodies are highly specific recombinant monoclonal antibodies developed by immunizing animals with the antigen, screening antibodies for desired functionality, and then cloning the immunogen-specific antibody genes into high-expression vectors. The antibodies are produced on a large scale by expressing them in mammalian cells, and then highly purified with protein A. These recombinant antibodies can be used just like traditional IgG antibodies but are designed to provide very consistent results from lot to lot, saving time and money because assays do not require revalidation.

SOX9 is a member of the SOX family of developmental transcription factors that are related to the Y-chromosome sex-determining factor

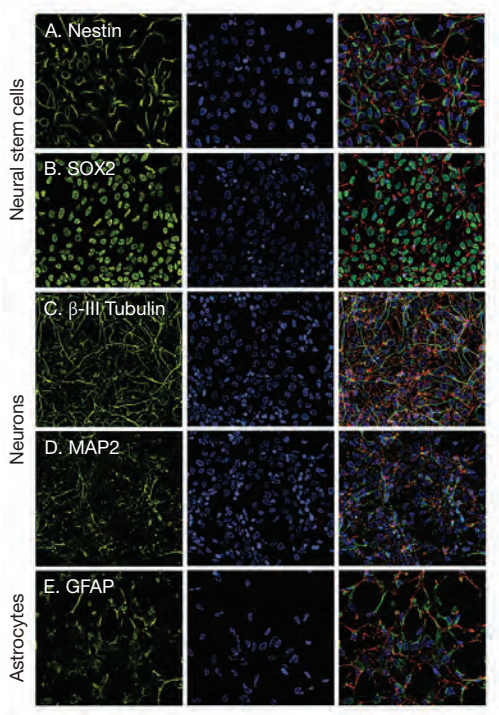


**Figure 1. Characterization of neural antibodies using differentiated embryonic stem cells.** Human neural stem cells (NSCs) differentiated from H9-derived embryonic stem cells using Gibco™ PSC Neural Induction Medium (Cat. No. A1647801) were used to characterize antibodies against neural progenitor markers **(A)** nestin (Cat. No. MA1110) and **(B)** SOX2 (Cat. No. MA1014). Differentiated neurons derived from NSCs using Gibco™ StemPro™ NSC SFM (Cat. No. A1050901) were used to characterize antibodies against mature neuronal markers **(C)** MAP2 (Cat. No. 131500) and **(D)** β-III tubulin (Cat. No. 322600); an antibody against **(E)** GFAP (Cat. No. MA512023) was characterized using differentiated astrocytes. Primary monoclonal antibodies were detected with Invitrogen™ Alexa Fluor™ 488 goat anti–mouse IgG secondary antibody (green, left column; Cat. No. A28175), nuclei were stained with DAPI (blue, middle column; Cat. No. D1306), and F-actin was labeled with rhodamine phalloidin (red, Cat. No. R415); right column shows composite images. Appropriate negative controls (MAP2 in neural stem cells and astrocytes, and nestin in neurons) were used to determine specificity (data not shown).

SRY. SOX9, which functions in the development of multiple organs, was recently reported to be involved in the early differentiation of ESCs into three germ layers [2]. The specificity of an ABfinity anti-SOX9 antibody was evaluated using the Gibco™ Human Episomal iPSC Line and the embryoid bodies (EBs) derived from them (Figure 2). As expected, nuclear localization of SOX9 was observed only in the EBs, which are predominantly composed of progenitors of all three germ layers, whereas expression was completely absent in the undifferentiated cells.

RUNX2 is a critical regulator of osteogenic development and plays an essential role in the specification of osteogenic lineage by inducing the expression of extracellular matrix proteins during maturation. Its expression during osteoblast differentiation is temporally controlled, peaking at day 7 and decreasing to undetectable levels by day 14 [3-5]. Human bone marrow–derived mesenchymal stem cells (MSCs) were differentiated to osteocytes using the Gibco™ StemPro™ Osteogenesis Differentiation Kit, and the specificity of an ABfinity anti-RUNX2 antibody was evaluated on cells throughout this differentiation process, from day 0 to day 14 (Figure 3). As expected, RUNX2 expression was absent in undifferentiated MSCs, specifically localized to the early osteoblast at day 7, and then undetectable in the mature osteoblast at day 14.

## Find your stem cell antibody

Advances in the field of stem cell therapy are critically dependent on the availability of highly specific antibodies that have been validated in biologically relevant model systems; find out more about our stringent antibody validation* criteria at **thermofisher.com/antibodyvalidation**. No matter which detection platform you use—flow cytometry, immunocytochemistry, western blot, or ELISA—our collection of over 51,000 Invitrogen™ antibodies provides you with tools compatible with your experimental design. Select the right antibodies for your stem cell targets at **thermofisher.com/antibodiesbp77**. ■

*The use or any variation of the word "validation" refers only to research-use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product or products were validated for clinical or diagnostic uses.

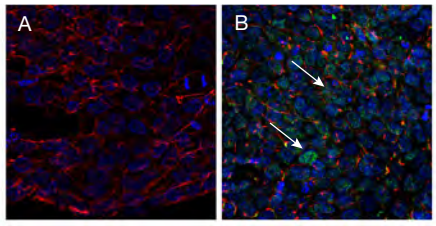


**Figure 2. Acceleration by SOX9 of the differentiation of pluripotent stem cells to progenitors of all three lineages.** Cells from the Gibco™ Human Episomal iPSC Line (Cat. No. A18945) labeled with anti-SOX9 antibody (Cat. No. 702016) and Invitrogen™ Alexa Fluor™ 488 goat anti–rabbit IgG secondary antibody (green, Cat. No. A27034) revealed that SOX9 is **(A)** absent in the human episomal iPS colony but **(B)** present in embryoid bodies (EBs), which are derived from the iPSC line and have progenitors of all three lineages (arrows). Nuclei were stained with DAPI (blue, Cat. No. D1306); F-actin was labeled with rhodamine phalloidin (red, Cat. No. R415).

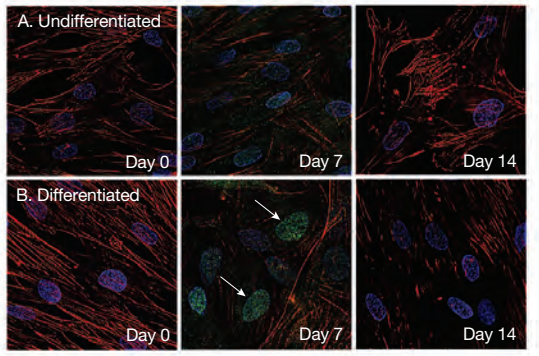


**Figure 3. Activation by RUNX2 of osteoblast differentiation in human bone marrow–derived mesenchymal stem cells.** Temporal RUNX2 expression at day 0, 7, and 14 in **(A)** undifferentiated human bone marrow–derived mesenchymal cells and **(B)** those subjected to osteoblast differentiation using the Gibco™ StemPro™ Osteogenesis Differentiation Kit (Cat. No. A1007201), as revealed by immunostaining with anti-RUNX2 antibody (Cat. No. 711519) and Invitrogen™ Alexa Fluor™ 488 goat anti–rabbit IgG secondary antibody (green, Cat. No. A27034) (arrows). Nuclei were stained with DAPI (blue, Cat. No. D1306); F-actin was labeled with rhodamine phalloidin (red, Cat. No. R415).

| Product | Quantity | Cat. No. |
|---|---|---|
| **Cells and media** | | |
| Human Episomal iPSC Line | 1 x $10^6$ cells | A18945 |
| PCS Neural Induction Medium | 500 mL | A1647801 |
| StemPro™ Neural Stem Cells | 1 x $10^6$ cells | A15654 |
| StemPro™ NSC SFM | 1 kit | A1050901 |
| StemPro™ Osteogenesis Differentiation Kit | 1 kit | A1007201 |
| **Selected stem cell antibodies** | | |
| GFAP Monoclonal Antibody (ASTRO6) | 500 µL | MA512023 |
| MAP2 Monoclonal Antibody (M13) | 100 µg | 131500 |
| Nestin Monoclonal Antibody (10C2) | 100 µg | MA1110 |
| RUNX2 ABfinity™ Rabbit Oligoclonal Antibody (6HCLC) | 100 µg | 711519 |
| SOX2 Monoclonal Antibody (20G5) | 100 µg | MA1014 |
| SOX9 ABfinity™ Rabbit Monoclonal Antibody (7H13L8) | 100 µg | 702016 |
| β-III Tubulin Monoclonal Antibody (2 28 33) | 100 µg | 322600 |

### References

1. Wu J, Izpisua Belmonte JC (2016) *Cell* 165:1572–1585.
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4. Maruyama Z, Yoshida CA, Furuichi T et al. (2007) *Dev Dyn* 236:1876–1890.
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