# STEMdiff™ Human Neural Progenitor Antibody Panel

**Antibody panel for the identification of neural progenitor cells and undifferentiated human ES/iPS cells**

Catalog #69001 1 Kit

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## Product Description

STEMdiff™ Human Neural Progenitor Antibody Panel is suitable for immunocytochemical characterization of neural progenitor cells, including those generated from human embryonic stem (ES) and induced pluripotent stem (iPS) cells. The panel provides primary antibodies that are immunoreactive towards marker proteins highly expressed either by neural progenitor cells (Nestin, PAX6, SOX1) or by undifferentiated human ES and iPS cells (OCT4/OCT3). This panel may be used to monitor the generation of neural progenitor cells during differentiation of pluripotent stem cells and is also suitable for labeling primary cells and tissues. All antibodies have been validated for immunocytochemistry. Antibodies against Nestin, OCT4 (OCT3), and PAX6 may also be used for flow cytometry.

## Product Information

| PRODUCT NAME | CATALOG # | QUANTITY | UNIT SIZE | DOCUMENT # |
|---|---|---|---|---|
| Anti-Human Nestin Antibody, Clone 10C2 | 60091 | 1 | 100 µg | 27682 |
| Anti-Human OCT4 (OCT3) Antibody, Clone 3A2A20 | 60093 | 1 | 100 µg | 27684 |
| Anti-PAX6 Antibody* | 60094 | 1 | 0.1 mL | 27685 |
| Anti-Human SOX1 Antibody, Clone EPR4766* | 60095 | 1 | 0.1 mL | 28686 |

*This component is sold as part of the STEMdiff™ Human Neural Progenitor Antibody Panel (Catalog #69001) and is not available for individual sale.

NOTE: For additional information, including storage instructions, refer to the appropriate Product Information Sheet (see Document # in the table above).

## Directions for Use

NOTE: This protocol is recommended for labeling of fixed cells for immunocytochemistry.

1. Optional step: Wash samples 3 times with phosphate-buffered saline (PBS).
2. Fix samples with 4% paraformaldehyde in PBS. Incubate at room temperature (15 - 25°C) for 15 minutes.
3. Wash samples with PBS (3 x 15 minutes) at room temperature.
4. Permeabilize samples by incubation in PBS + 0.1% Triton™ X-100 (or PBS + 0.1% TWEEN® 20) at room temperature for 10 minutes.
5. Remove permeabilization buffer and replace with blocking solution (PBS + 5% serum). Incubate at room temperature for 60 minutes.
   NOTE: Do not use serum obtained from the same species used to generate the primary antibodies.
6. Prepare working stocks of primary antibodies by diluting in blocking solution. See Table 1 for recommended working dilutions.
   NOTE: The PAX6 and SOX1 antibodies cannot be used to simultaneously label the same sample.

**Table 1. Recommended Working Dilutions of Primary Antibodies**

| TARGETED ANTIGEN | HOST SPECIES | ISOTYPE | CATALOG # | WORKING DILUTION* |
|---|---|---|---|---|
| Nestin | Mouse | IgG1, kappa | 60091 | 1 in 2000 |
| OCT4 (OCT3) | Mouse | IgG2b, kappa | 60093 | 1 in 1000 |
| PAX6 | Rabbit | IgG (polyclonal) | 60094 | 1 in 500 |
| SOX1 | Rabbit | IgG | 60095 | 1 in 1000 |

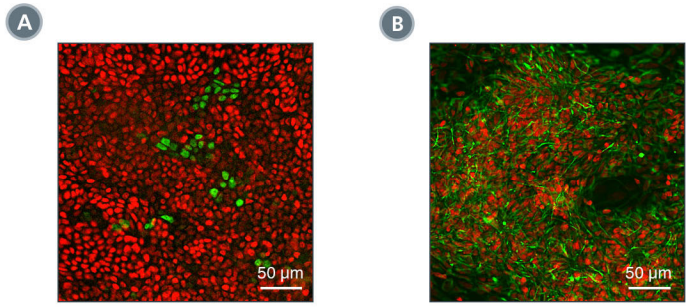
*Each primary antibody should be titrated for optimal performance.

7. Remove blocking solution and add primary antibodies. Incubate at room temperature (15 - 25°C) for 3 - 5 hours or at 2 - 8°C overnight.
8. Wash samples with PBS (3 x 15 minutes) at room temperature.

9. Aspirate PBS and add secondary antibodies diluted in blocking solution. Incubate at room temperature for 60 minutes.

   NOTE: Fluorochromes conjugated to secondary antibodies are sensitive to light; where possible, protect from prolonged exposure to light.

10. Wash samples with PBS (3 x 15 minutes) at room temperature.




(A) WLS-4D1 (iPS) cells maintained in TeSR™-E8™ (Catalog #05940) were dissociated, plated as single cells, and cultured in STEMdiff™ Neural Induction Medium (Catalog #05835) for differentiation into neural progenitor cells. Cells were fixed after 7 days in culture and labeled with Anti-PAX6 Antibody and Anti-Human OCT4 (OCT3) Antibody, Clone 3A2A20, followed by fluorochrome-conjugated secondary antibodies. Cells that have adopted a neural fate are positive for PAX6 (red) whereas undifferentiated iPS cells are positive for OCT4 (OCT3) (green).

(B) Neural progenitor cells derived from H9 (ES) cells using STEMdiff™ Neural Induction Medium were cultured in STEMdiff™ Neural Progenitor Medium (Catalog #05833). Cells were fixed and labeled with Anti-Human SOX1 Antibody, Clone EPR4766, and Anti-Human Nestin, Clone 10C2, followed by fluorochrome-conjugated secondary antibodies. Cells positive for the neural progenitor cell markers SOX1 (red) and Nestin (green) are evident.