NEUROMICS

Nestin Data Sheet

Catalog Number:	MO15012	**Host:**	Mouse
Product Type:	Mouse Monoclonal IgG1 Protein G purified Cell Culture Supernatant. Clone: 196908	**Species Reactivity:**	Human, Primate
Immunogen Sequence:	human Nestin fragment (amino acid residues 618 - 1618) expressing NSO cells	**Format:**	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose.

Applications: Immunocytochemistry: 5-10 µg/mL
Flow Cytometry-see: PLoS ONE 8(6): e68519. doi:10.1371/journal.pone.0068519.
Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Avoid repeated freeze-thaw cycles.

References:

Turaç G, Hindley CJ, Thomas R, Davis JA, Deleidi M, et al. (2013) Combined Flow Cytometric Analysis of Surface and Intracellular Antigens Reveals Surface Molecule Markers of Human Neuropoiesis. PLoS ONE 8(6): e68519. doi:10.1371/journal.pone.0068519.

Fabien G. Lafaille, Itai M. Pessach, Shen-Ying Zhang, Michael J. Ciancanelli, Melina Herman, Avinash Abhyankar, Shui-Wang Ying, Sotirios Keros, Peter A. Goldstein, Gustavo Mostoslavsky, Jose Ordovas-Montanes, Emmanuelle Jouanguy, Sabine Plancoulaine, Edmund Tu, Yechiel Elkabetz, Saleh Al-Muhsen, Marc Tardieu, Thorsten M. Schlaeger, George Q. Daley, Laurent Abel, Jean-Laurent Casanova, Lorenz Studer, Luigi D. Notarangelo. Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. Nature (2012) doi:10.1038/nature11583.

J. Simon Lunn, Crystal Pacut, Emily Stern, Stacey A. Sakowski, J. Matthew Velke, Sue O'Shea, Eva L. Feldman. Intraspinal transplantation of neurogenin-expressing stem cells generates spinal cord neural progenitors. Neurobiology of Disease. Volume 46, Issue 1, April 2012, Pages 59–68.

Application Notes

Immunocytochemistry
This antibody can be used with the appropriate secondary reagents at the concentration of 5 - 10 µg/mL in fixed cells. Cells were fixed with 4% paraformaldehyde and 0.15% picric acid in PBS at room temperature for 20 min., followed by blocking with PBS containing 10% normal donkey serum and 1% BSA at room temperature for 45 min. After blocking, cells were incubated with diluted primary antibody overnight at 4° C and followed by incubation for 1 hour with appropriate secondary antibody at room temperature. Between each step, cells were washed with PBS and 0.1% BSA.

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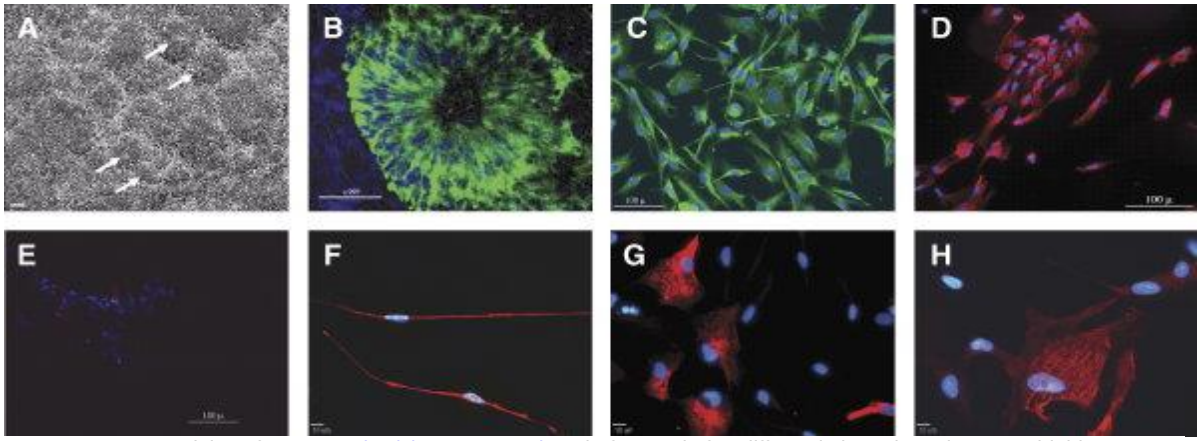
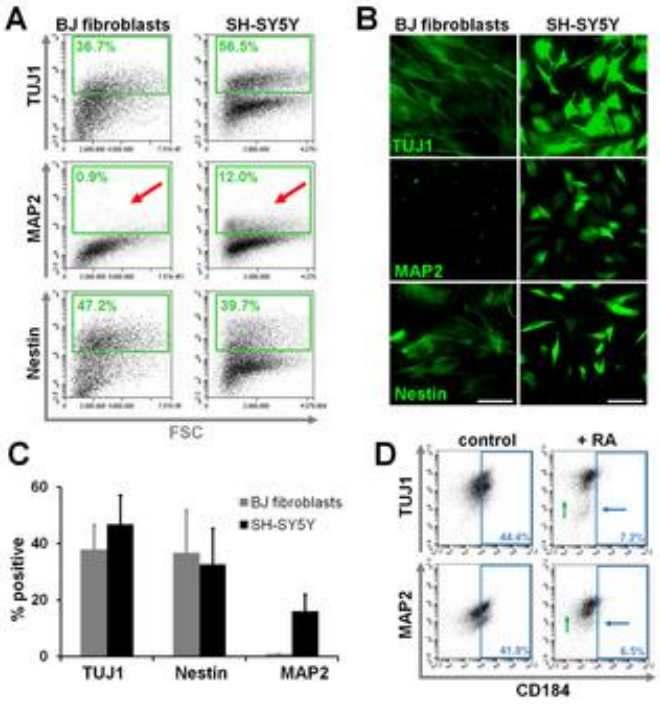


Images: Immunostaining of STEMEZ(TM) hNP1 Progenitors before and after differentiation. The culture was highly homogenous with neural rosettes. (A) Neural rosettes (white arrows, bright field) from WA09 cells. A similar result also obtained with BG02 cell line. (B) Shown here, a neural rosette stained with Nestin (NES) (green) antibody. Propagated Neural progenitors showed expression of marker genes, NES (C) and Musashi 1 (D) but not SOX2 (E). Further differentiation produced neurons (Tuj1) (F), astrocytes (GFAP) (G) and oligodendrocytes (myelin basic protein)(H) (lower panel). DAPI (blue) was used for staining the nuclei (scale bar for (A) through (E) is 100 mm and for the remaining figures 10 mm). Differentiation (2007) DOI: 10.1111/j.1432-0436.2007.00256.x...Dilutions: NES (1:100, Neuromics, Edina, MN), MSI1 (1:100, Neuromics), Tuj1 (1:500, Neuromics)...

Images: Accurate detection of intracellular antigens with optimized fixation-permeabilization conditions preserving surface antigens. Flow cytometric detection of TUJ1, MAP2 and nestin antigens in BJ fibroblasts and the neural SH-SY5Y cell line (A). TUJ1 and nestin are present in both cell lines, while the mature neuronal marker MAP2 was only detected in SH-SY5Y cells (arrows). Note stable fluorescent levels of the negative population, indicating low background staining using this protocol. Representative experiment of three independent repeats shown. (B) Corresponding validation by immunofluorescence analysis. (C) Quantitation of TUJ1, MAP2 and nestin intracellular antigen detection (n=3). Error bars indicate standard deviation. (D) Response of TUJ1 and MAP2 intracellular antigen expression to 6 DIV of 10 µM retinoic acid (RA) treatment of SH-SY5Y cells. Note disappearance/reduction of subsets negative for these markers (upward shift, green arrows), as well as a shift toward CD184low expression with differentiation (blue arrows). doi:10.1371/journal.pone.0068519.g003



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