

Human Neural Stem Cell Immunocytochemistry Kit

Catalog no. A24354

Table 1 Contents and storage

Kit component	Part no.	Concentration	Amount	Storage	Usage notes
Primary antibodies					
anti-NESTIN (host: mouse)	A24345	50X	20 µL	-20°C to 4°C	Dilute with Blocking Solution
anti-PAX6 (host: rabbit)	A24340				
anti-SOX1 (host: goat)	A24347				
anti-SOX2 (host: rabbit)	A24339				
Secondary antibodies					
Alexa Fluor® 488 donkey anti-mouse; for use with anti-NESTIN	A24350	250X	20 µL	-20°C to 4°C; avoid freeze-thaw cycles	Ex/Em ¹ 495/519 nm (green); spin before use ²
Alexa Fluor® 488 donkey anti-goat; for use with anti-SOX1	A24349				Ex/Em ¹ 495/519 nm (green); spin before use ²
Alexa Fluor® 555 donkey anti-rabbit; for use with anti-PAX6 or anti-SOX2	A24342		40 µL		Ex/Em ¹ 555/565 nm (orange); spin before use ²
Alexa Fluor® 594 donkey anti-rabbit; for use with anti-PAX6 or anti-SOX2	A24343				Ex/Em ¹ 590/617 nm (red); spin before use ²
Additional reagents					
NucBlue® Fixed Cell Stain (DAPI nuclear DNA stain)	R37606	NA	1 vial	-20°C to ambient temperature	Ex/Em ¹ 358/461 nm (blue); apply 2 drops/mL
Fixative Solution	A24344	1X	10 mL		4% formaldehyde in DPBS
Permeabilization Solution	A24352		0.5% Triton® X-100 in DPBS		
Blocking Solution	A24353		20 mL		3% BSA in DPBS
Wash Buffer	A24348	10X			Dilute to 1X with water

Handling and shelf life: Use aseptic technique when handling all reagents. Allow frozen reagents to thaw completely before using them. Once thawed, the kit should not be refrozen (aliquots not recommended). Store at 4°C for up to 6 months.

¹ Approximate excitation/emission wavelength maxima.

² Centrifuge Secondary Antibody solutions (e.g., 2 minutes at 10,000 × g) and add only the supernatant to the Blocking Solution. This step eliminates any protein aggregates that may have formed during storage, thereby reducing non-specific background staining.

Description

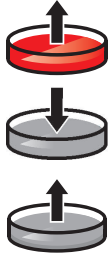
This kit contains a complete set of primary and secondary antibodies along with ready-to-use buffers to enable facile immunocytochemistry characterization of human neural stem cells (NSC). The primary antibodies included in this kit target well-established NSC markers (Nestin, PAX6, SOX1, and SOX2) and were carefully selected to help ensure excellent performance in immunocytochemistry applications.

For Research Use Only. Not for use in diagnostic procedures.

Experimental protocol

See Table 2, page 3, for recommended volumes to use based on the culture format of the cells to be stained. See Table 3, page 3, for multiplex staining options.

Caution: Use gentle liquid handling and pipeting techniques when adding or removing liquids to minimize the possibility of dislodging cells and losing them during the handling steps.



1. Remove media from the cells.



2. Add Fixative Solution and incubate for 15 minutes at room temperature.



3. Remove Fixative Solution.

Optional stopping point: After removing Fixative, add Wash Buffer (diluted to 1X with water), wrap the sample to prevent it from drying out, and store at 4°C for up to 2 weeks.



4. Add Permeabilization Solution and incubate 15 minutes at room temperature.



5. Remove Permeabilization Solution.



6. Add Blocking Solution and incubate 1 hour at room temperature.



7. Remove Blocking Solution.



8. Add desired Primary Antibody (diluted to 1X in Blocking Solution; see Table 3 for options) and incubate for 3 hours at room temperature (or overnight at 4°C).



9. Wash 3 times with Wash Buffer (diluted to 1X with water).



10. Add the appropriate Secondary Antibody (diluted to 1X in Blocking Solution; see Table 3 for guidance) and incubate for 1 hour at room temperature.



11. Wash 3 times with Wash Buffer (diluted to 1X with water).

Optional: Add 2 drops/mL of NucBlue® Fixed Cell Stain (DAPI) into the last wash step and incubate for 5 minutes.



12. Image the cells immediately. Cells can also be stored at 4°C in the dark for up to 1 week.

Table 2 Recommended final volumes to use during the protocol.

Culture format	Volume	No. of tests ¹
96-well plate	50 µL/well	20
48-well plate	100 µL/well	10
24-well plate	200 µL/well	5
12-well plate	400 µL/well	2
6-well plate	1000 µL/well	1
35-mm dish	1000 µL/dish	1
4-well chamber slide	400 µL/well	2
8-well chamber slide	200 µL/well	5

¹ When using the suggested staining volume, this kit contains sufficient reagents for the indicated number of tests per primary antibody.

Table 3 Dual antibody staining options. Note that the NucBlue® Fixed Cell Stain (a DAPI nuclear DNA stain) provided in this kit is also compatible with these antibody combinations. See Figure 1, below, for example pictures.

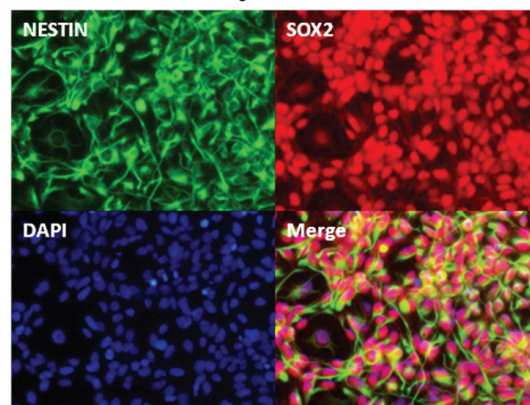
Color options:	Green ¹ (e.g., FITC filter)	Orange ¹ (e.g., Cy ³ / TRITC filter) or Red ¹ (e.g., Texas Red® filter)
Antibody combination # 1: NESTIN + SOX2		
Primary antibody	anti-NESTIN (host: mouse)	anti-SOX2 (host: rabbit)
Secondary antibody	Alexa Fluor® 488 donkey anti-mouse	Alexa Fluor® 555 donkey anti-rabbit or Alexa Fluor® 594 donkey anti-rabbit
Antibody combination # 2: SOX1 + PAX6		
Primary antibody	anti-SOX1 (host: goat)	anti-PAX6 (host: rabbit)
Secondary antibody	Alexa Fluor® 488 donkey anti-goat	Alexa Fluor® 555 donkey anti-rabbit or Alexa Fluor® 594 donkey anti-rabbit

¹ See Table 1, page 1, for approximate excitation/emission wavelength maxima.

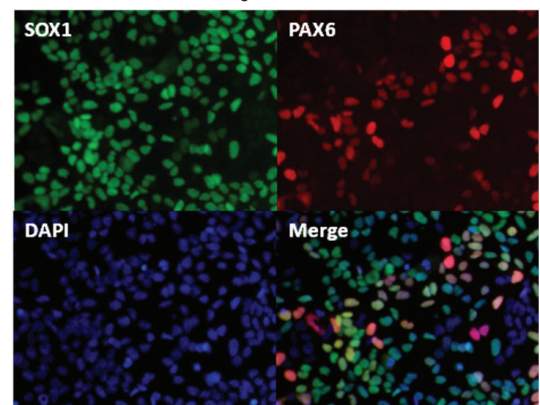
Figure 1 Neural stem cells derived from an iPSC line using Gibco® PSC Neural Induction Medium (Cat. no. A1647801) were stained for NSC markers Nestin and SOX2 (antibody combination #1) or SOX1 and PAX6 (antibody combination #2) and nuclear DNA (DAPI) using the Human Neural Stem Cell Immunocytochemistry Kit (Cat. no. A24354).

Note that not all NSCs will stain positive for PAX6, this is normal and to be expected [i.e., only NSCs that possess forebrain neuron fate commitment will stain positive]. In our experience, approximately 15–50% of NSCs generated using Gibco® PSC Neural Induction Medium stain positive for PAX6.

Antibody combination # 1: NESTIN + SOX2 with additional DAPI (nuclear DNA) staining.



Antibody combination # 2: SOX1 + PAX6 with additional DAPI (nuclear DNA) staining.



Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
A24354	Human Neural Stem Cell Immunocytochemistry Kit	1 kit
Related Products		
A1647801	Gibco® PSC Neural Induction Medium	1 kit
N7800100	Gibco® Human Neural Stem Cells (H9-Derived)	1 mL
R37602	Image-iT® Fixation/Permeabilization Kit	1 kit
A13998	OCT4 Rabbit Monoclonal Antibody (clone C30A3)*	100 µL
	*Pluripotent marker OCT4 is a good negative control marker for NSCs	

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Additional international offices are listed at
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These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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