

Human Cardiomyocyte Immunocytochemistry Kit

Catalog no. A25973

Table 1 Contents and storage

Kit component	Part no.	Concentration	Amount	Storage	Usage notes
Primary antibodies					
anti-NKX2-5 (host: rabbit)	A25974	1000X	10 µL	-20°C to 4°C	Dilute with Blocking Solution
anti-TNNT2 (host: mouse)	A25969				
Secondary antibodies					
Alexa Fluor® 555 donkey anti-rabbit; for use with anti-NKX2-5	A25971	250X	40 µL	-20°C to 4°C; avoid freeze-thaw cycles	Ex/Em ¹ 555/565 nm (orange); spin before use ²
Alexa Fluor® 594 donkey anti-rabbit; for use with anti-NKX2-5	A25970				Ex/Em ¹ 590/617 nm (red); spin before use ²
Alexa Fluor® 488 donkey anti-mouse; for use with anti-TNNT2	A25972				Ex/Em ¹ 495/519 nm (green); 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 1–2 drops/mL
Fixative Solution	A24344	1X	10 mL		4% formaldehyde in DPBS
Permeabilization Solution S	A24878		1% Saponin in DPBS		
Blocking Solution	A24353		3% BSA in DPBS		
Wash Buffer	A24348	10X	20 mL		Dilute to 1X with water ³

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.

³ Upon thawing the 10X Wash Buffer, you may observe a precipitate that should go back into solution when warmed to ambient temperature and mixed well.

Description

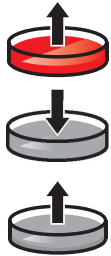
The Human Cardiomyocyte Immunocytochemistry Kit enables optimal image-based analysis of two key markers of the human cardiac lineage: NKX2-5 for early cardiac mesoderm and TNNT2/cTNT for cardiomyocytes. This high performance immunocytochemistry (ICC) kit includes a complete set of primary and secondary antibodies, a nuclear DNA stain, and pre-made buffers for an optimized staining experiment.

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 the Fixative solution, add Wash Buffer (diluted to 1X with water), wrap the sample in Parafilm[®] laboratory film to prevent it from drying out, and store at 4°C for up to 1 month.



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



5. Remove Permeabilization Solution.



6. Add Blocking Solution and incubate 30 minutes at room temperature.



7. Add desired Primary Antibody (see Table 3 for co-staining options) directly to the Blocking Solution covering the cells to yield a 1X final dilution. Mix gently and incubate for 3 hours at room temperature (or overnight at 4°C).



8. Remove the solution. Add Wash Buffer (diluted to 1X with water) and incubate for 2–3 minutes. Repeat the wash procedure 2 more times so that the cells are washed a total of 3 times.



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



10. Remove the solution. Add Wash Buffer (diluted to 1X with water) and wait for 2–3 minutes. Repeat the wash procedure 2 more times so that the cells are washed a total of 3 times.

Optional: Add 1–2 drops/mL of NucBlue[®] Fixed Cell Stain (DAPI) into the last wash step and incubate for 5 minutes.



11. Image the cells immediately or store cells at 4°C in the dark, wrapped with Parafilm[®] laboratory film to prevent the samples from drying out, for up to 1 month.

Alternatively, for prolonged storage, apply a suitable antifade mounting medium, such as ProLong[®] Diamond Antifade Mountant, to the sample.

Table 2 Recommended final volumes to use during the protocol.

Culture format	No. of tests ¹	Staining volume	Amount of each 1000X primary antibody to add ²	Amount of each 250X secondary antibody to add
96-well plate	200	50 µL/well	0.05 µL	0.2 µL
48-well plate	100	100 µL/well	0.1 µL	0.4 µL
24-well plate	50	200 µL/well	0.2 µL	0.8 µL
12-well plate	25	400 µL/well	0.4 µL	1.6 µL
6-well plate	10	1000 µL/well	1 µL	4 µL
35-mm dish	10	1000 µL/dish	1 µL	4 µL
4-well chamber slide	25	400 µL/well	0.4 µL	1.6 µL
8-well chamber slide	50	200 µL/well	0.2 µL	0.8 µL

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

² To avoid working with very small volumes, first prepare a 10X working dilution (e.g., add 1 µL of each 1000X primary antibody to 100 µL of Blocking Solution) and then dispense a 1/10 volume (e.g., add 5 µL to 45 µL in the well) to dilute to a 1X final concentration.

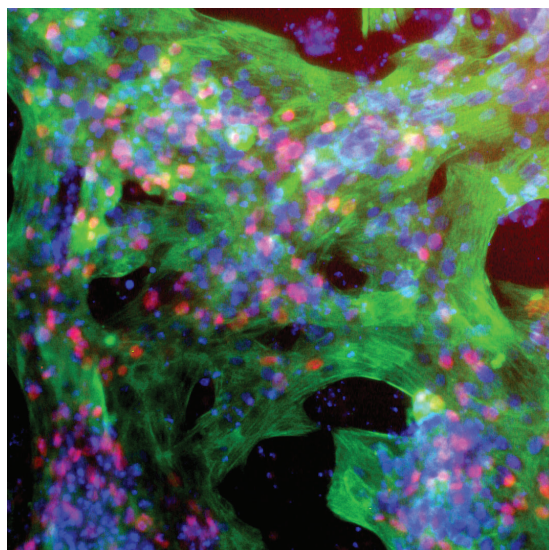
Table 3 Antibody co-staining options. Note that the NucBlue® Fixed Cell Stain (a DAPI nuclear DNA stain) provided in this kit is also compatible with these antibody combinations. See Figure 1, below, for example pictures.

Color options:	Green ¹ (e.g., FITC filter)	Orange ¹ (e.g., Cy ³ / TRITC filter) or Red ¹ (e.g., Texas Red® filter)
Primary antibody	anti-TNNT2 (host: mouse)	anti-NKX2-5 (host: rabbit)
Secondary antibody	Alexa Fluor® 488 donkey anti-mouse	Alexa Fluor® 555 donkey anti-rabbit or Alexa Fluor® 594 donkey anti-rabbit

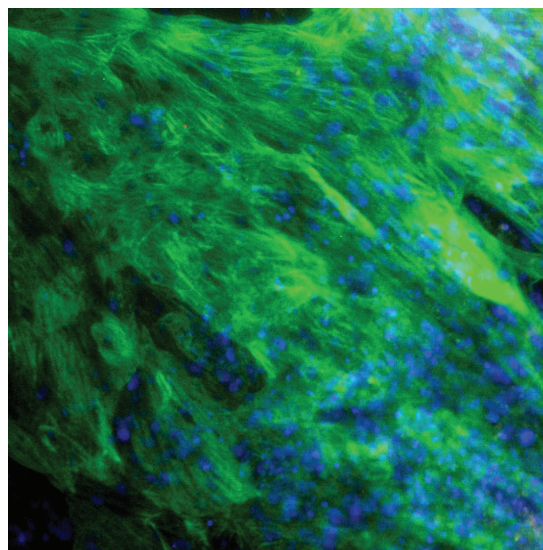
¹ See Table 1, page 1, for approximate excitation/emission wavelength maxima.

Figure 1 Induced pluripotent stem cells (iPSCs) were differentiated using the PSC Cardiomyocyte Differentiation Kit (Cat. no. A25042SA) for 14 or 67 days prior to staining for the following markers with the Human Cardiomyocyte Immunocytochemistry Kit (Cat. no. A25973): NKX2-5 for early cardiac mesoderm and TNNT2/cTNT for cardiomyocytes.

Day 14 post-differentiation



Day 67 post-differentiation



Product List

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

Cat. no.	Product Name	Unit Size
A25973	Human Cardiomyocyte Immunocytochemistry Kit	1 kit
<i>Related Products</i>		
A24354	Human Neural Stem Cell Immunocytochemistry Kit	1 kit
A24881	PSC 4-Marker Immunocytochemistry Kit	1 kit
A25525	PSC (SOX2, TRA-1-60) Immunocytochemistry Kit	1 kit
A25526	PSC (OCT4, SSEA4) Immunocytochemistry Kit	1 kit
A25538	3-Germ Layer Immunocytochemistry Kit	1 kit
P36965	ProLong [®] Diamond Antifade Mountant	5 × 2 mL
A15871	TaqMan [®] hPSC Scorecard [™] Kit, FAST 96 well	2 plates
A14353	Alkaline Phosphatase Live Stain	50 µL
A18945	Gibco [®] Human Episomal iPSC Line	1 vial
A16517	CytoTune [®] -iPS 2.0 Sendai Reprogramming Kit	1 pack
A14703	Episomal iPSC Reprogramming Vectors	1 kit
A15960	Epi [™] Episomal iPSC Reprogramming Kit	1 kit
A1517001	Essential 8 [®] Medium	500 mL
A14700	Vitronectin (VTN-N) Recombinant Human Protein, Truncated	1 mL
A1647801	PSC Neural Induction Medium	500 mL
A25042SA	PSC Cardiomyocyte Differentiation Kit (Prototype)	1 kit

Purchaser Notification

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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