3-Germ Layer Immunocytochemistry Kit

Catalog Number A25538

Pub. No. MAN0010484 **Rev.** B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The 3-Germ Layer Immunocytochemistry Kit enables image-based analysis of spontaneously differentiated embryoid bodies derived from human pluripotent stem cells. It contains a complete set of primary and secondary antibodies along with premade buffers to enable convenient detection of widely accepted germ layer markers: beta-III tubulin (TUJ1) for ectoderm, alpha-fetoprotein (AFP) for endoderm, and smooth muscle actin (SMA) for mesoderm.

Contents and storage

Kit component	Part no.	Concentration	Amount	Storage	Usage notes
Primary antibodies					
anti-TUJ1 (host: rabbit)	A25532	500X	10 µL	-20°C to 4°C	Dilute with Blocking Solution
anti-AFP (host: mouse IgG1)	A25530				
anti-SMA (host: mouse IgG2a)	A25531	200X	20 µL		
Secondary antibodies					
Alexa Fluor™ 488 donkey anti-rabbit; for use with anti-TUJ1	A25535	250X	20 µL	-20°C to 4°C; avoid freeze-thaw cycles	Ex/Em* 495/519 nm (green); spin before use**
Alexa Fluor™ 647 donkey anti-rabbit; for use with anti-TUJ1	A25537				Ex/Em* 650/668 nm (far red); spin before use**
Alexa Fluor™ 488 goat anti-mouse IgG1; for use with anti-AFP	A25536				Ex/Em* 495/519 nm (green); spin before use**
Alexa Fluor™ 555 goat anti-mouse IgG2a; for use with anti-SMA	A25533				Ex/Em* 555/565 nm (orange); spin before use**
Alexa Fluor™ 594 goat anti-mouse IgG2a; for use with anti-SMA	A25534				Ex/Em* 590/617 nm (red); spin before use**
Additional reagents					
NucBlue™ Fixed Cell Stain (DAPI nuclear DNA stain)	R37606	NA	1 vial	-20°C to ambient temperature	Ex/Em* 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		20 mL		3% BSA in DPBS
Wash Buffer	A24348	10X			10X DPBS, 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, do not re-freeze the kit (aliquots are not recommended). Store at 2°C to 8°C for up to 6 months.

* Approximate excitation/emission wavelength maxima.

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

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

Perform experiment

See Table 1 below for recommended volumes. See Table 2 for multiplex staining options.

CAUTION! Use care when adding or removing liquids to minimize the possibility of dislodging the cells.

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), parafilm the sample 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 2 for co-staining options) directly to the Blocking Solution covering the cells to yield a 1X final dilution, mix gently, and incubate overnight at 4°C.
8. 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.
9. Remove the third Wash Buffer and add the appropriate Secondary Antibody (diluted to 1X in Blocking Solution; see Table 2 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 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 1 Recommended final volumes to use during the protocol.

Culture format	No. of tests*	Staining volume	Amount of 500X anti-TUJ1 or AFP to add	Amount of 200X anti-SMA to add	Amount of each 250X secondary antibody to add
96-well plate	80	50 µL/well	0.1 µL	0.25 µL	0.2 µL
48-well plate	40	100 µL/well	0.2 µL	0.50 µL	0.4 µL
24-well plate	20	200 µL/well	0.4 µL	1 µL	0.8 µL
12-well plate	10	400 µL/well	0.8 µL	2 µL	1.6 µL
6-well plate	4	1,000 µL/well	2 µL	5 µL	4 µL
35-mm dish	4	1,000 µL/dish	2 µL	5 µL	4 µL
4-well chamber slide	10	400 µL/well	0.8 µL	2 µL	1.6 µL
8-well chamber slide	20	200 µL/well	0.4 µL	1 µL	0.8 µL
* When using the suggested staining volume, this kit contains sufficient reagents for the indicated number of tests per primary antibody.					

Table 2 Multiplex 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 for example pictures.

Color options	Green* (e.g., FITC filter)	Orange* (e.g., Cy®3/TRITC filter) or Red* (e.g., Texas Red™ filter	Far red*
Antibody combination # 1: AFP + SMA + TUJ1			
Primary antibody	anti-AFP (host: mouse IgG1)	anti-SMA (host: mouse IgG2a)	Anti -TUJ1 (host: rabbit)
Secondary antibody	Alexa Fluor™ 488 goat anti-mouse IgG1	Alexa Fluor™ 555 goat anti-mouse IgG2a or Alexa Fluor™ 594 goat anti-mouse IgG2a	Alexa Fluor™ 647 donkey anti-rabbit
Antibody combination # 2: AFP + SMA			
Primary antibody	anti-AFP (host: mouse IgG1)	anti-SMA (host: mouse IgG2a)	—
Secondary antibody	Alexa Fluor™ 488 goat anti-mouse IgG1	Alexa Fluor™ 555 goat anti-mouse IgG2a or Alexa Fluor™ 594 goat anti-mouse IgG2a	
Antibody combination # 3: AFP +TUJ1			
Primary antibody	anti-AFP (host: mouse IgG1)	—	Anti -TUJ1 (host: rabbit)
Secondary antibody	Alexa Fluor™ 488 goat anti-mouse IgG1		Alexa Fluor™ 647 donkey anti-rabbit
Antibody combination # 4: TUJ1 + SMA			
Primary antibody	anti-TUJ1 (host: rabbit)	anti-SMA (host: mouse IgG2a)	—
Secondary antibody	Alexa Fluor™ 488 donkey anti-rabbit	Alexa Fluor™ 555 goat anti-mouse IgG2a or Alexa Fluor™ 594 goat anti-mouse IgG2a	
* See Table 1 for approximate excitation/emission wavelength maxima.			

Example data

Embryoid bodies generated from H9 stem cells (combinations 1–3) or iPSCs (combination 4) were allowed to randomly differentiate for 14–20 days. The cells were stained for the following embryonic germ layer markers using the 3-Germ Layer ICC Kit (Cat. no. A25538): endoderm marker alpha-fetoprotein (AFP), mesoderm marker smooth muscle actin (SMA), or ectoderm marker beta-III tubulin (TUJ1).

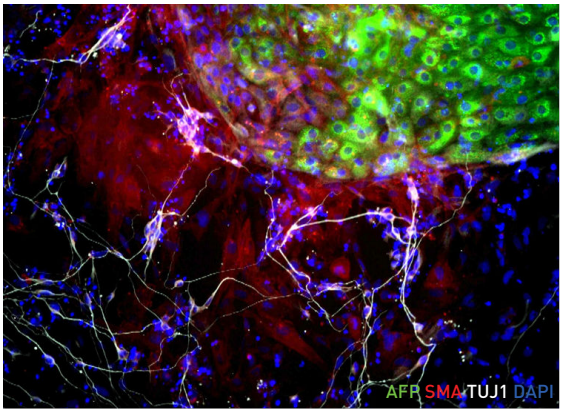

Fig. 1 Antibody combination 1: AFP + SMA + TUJ1 with additional DAPI (nuclear DNA) staining.

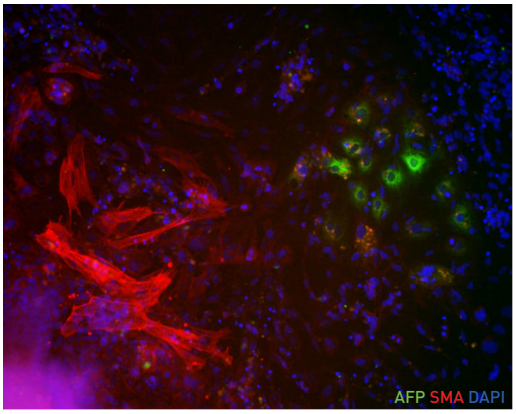

Fig. 2 Antibody combination 2: AFP + SMA with additional DAPI (nuclear DNA) staining.

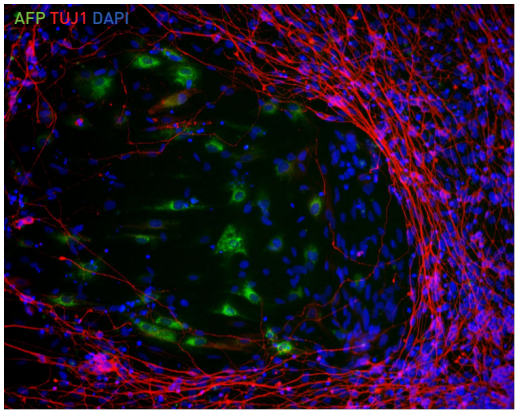

Fig. 3 Antibody combination 3: AFP + TUJ1 with additional DAPI (nuclear DNA) staining.

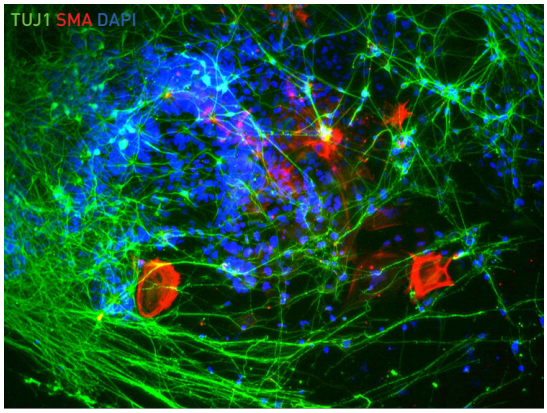

Fig. 4 Antibody combination 4: TUJ1 + SMA with additional DAPI (nuclear DNA) staining.

Related products

Product	Cat. no.
PSC 4-Marker Immunocytochemistry Kit	A24881
PSC (OCT4, SSEA4) Immunocytochemistry Kit	A25526
ProLong™ Diamond Antifade Mountant	P36965
TaqMan® hPSC Scorecard™ Kit, FAST 96 well	A15871
Alkaline Phosphatase Live Stain	A14353
Gibco™ Human Episomal iPSC Line	A18945
CytoTune™-iPS 2.0 Sendai Reprogramming Kit	A16517
Episomal iPSC Reprogramming Vectors	A14703
Epi5™ Episomal iPSC Reprogramming Kit	A15960
Essential 8™ Medium	A1517001
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
Human Neural Stem Cell Immunocytochemistry Kit	A24354
PSC Neural Induction Medium	A1647801

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