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Colocalisation imaging of endogenous TMEM192 with lysosomal and mitochondria markers

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We use this protocol and it's working

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Abstract

Immunofluorescent (IF) microscopy is a powerful tool used in cellular and molecular biology to monitor the subcellular localisation of proteins. By combining the advantages of immunostaining and confocal light microscope, IF microscopy can be used to assess the colocalization of two or more proteins within the cell. Here, we describe a method that can be used to verify the correct localisation of endogenously expressed TMEM192, by assessing their colocalization with LAMP1 (a lysosomal marker) and ATPB1 (a mitochondrial marker). Furthermore, our data showed that the anti-TMEM192 antibody is compatible for immunofluorescence assay.

Attachments

Colocalisation imagi...

736KB

Materials

Materials:

1. Cell lines
- HEK293 **ATCC Catalog #**CRL-1573 , RRID:CVCL_0045)

2.Antibodies
- See Tables 1 and 2

Table 1: List of primary antibodies

	A	B	C	D
	Antibody	**Company**	**Cat. number**	**Host species**
	TMEM192	Abcam	Ab232600	Rabbit
	LAMP1	Santa Cruz	Sc-20011	Mouse
	ATPB	Abcam	Ab14730	Mouse

Table 2: List of fluorophore-conjugated secondary antibodies

	A	B	C	D	E
	Antibody	**Conjugated Fluorophore**	**Company**	**Cat. number**	**Host Species**
	anti-Mouse	Alexa 488	Invitrogen	A21206	Donkey
	anti-Rabbit	Alexa 594	Invitrogen	A11012	Goat

3. Media and Reagents

- Growth Media:

1. DMEM (Gibco™ #11960-085) **Gibco - Thermo Fisher Scientific Catalog #**11960085
2. 10% (v/v) Fetal Bovine Serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F7524 Batch# BCBW6817)
3. 1% L-Glutamine (200mM) **Thermo Fisher Scientific Catalog #**25030024
4. 1% Penicillin-Streptomycin **Gibco - Thermo Fisher Scientific Catalog #**15140122

- DPBS no calcium no magnesium **Gibco - Thermo Fisher Scientific** **Catalog #**14190169
- Bovine Serum Albumin Fraction V **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**10735094001
  Sodium azide **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S2002
  Poly-L-lysine **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P4832
  Hoechst 33342 **Thermo Fisher Scientific** **Catalog #**62249
- VECTASHIELD antifading Mounting media (Vector Laboratories, H1000)

4. Equipment

- Incubator with FPI-sensor system and display controller MB1 (BINDER GmbH. Model: CB150. Power Output: 1.40kW, 230V, 6.1 Amp)
- Leica TCS SP8 MP Multiphoton Microscope.
- See-saw rocker (VWR SSL4, or equivalent).

5. Consumables

- Nunc™ Cell-Culture Treated Multidishes, 6 well **Thermo Fisher** **Catalog #**140675 .
- Cover glasses square **VWR International (Avantor)** **Catalog #**631-0125
- Microscope slides SuperFrost® **VWR International (Avantor)** **Catalog #**631-0114)
- Standard 1ml and 200µl Pipette tips (Greiner bio-one. Catalog# 686271 and 685261 respectively).

Troubleshooting

Seeding cells for immunofluorescence microscopy

1h

1 Coat coverslips (sterilised in 100% ethanol prior to use) with poly-L-lysine by immersing the coverslips in poly-L-lysine solution for 01:00:00 . 1h

2 Rinse the coated coverslips in media and place in a 6-well plate (one coverslip in each well).

3 Seed cells to 50-60% confluency in growth media on coated coverslips from step 2.

4 Incubate Overnight .

Preparing cells for Immunofluorescence imaging

3h 25m

5 Remove media completely using an aspirator and wash cells 3 times with 3 mL PBS added with 0.2% (w/v) BSA and 0.02% (w/v) sodium azide (00:05:00 per wash on a see-saw rocker). 5m

6 Fix cells by adding 4% (w/v) PFA in PBS and Incubate at Room temperature for 00:10:00 . 10m

7 Permeabilise cells with 1% (v/v) NP-40 in PBS + 0.2% (w/v) BSA + 0.02% (w/v) sodium azide.

8 Block with 3% (w/v) BSA in PBS at Room temperature for 00:30:00 . 30m

9 Prepare the primary antibody dilutions in 0.2% BSA (w/v) in PBS + 0.02% (w/v) sodium azide (See Table 1 for a list of antibodies and their working dilution).

	A	B	C	D	E
	Antibody	**Company**	**Cat. number**	**Host Species**	**dilution**

	A	B	C	D	E
	TMEM192	Abcam	Ab185545	Rabbit	1:1000
	LAMP1	Santa Cruz	Sc-20011	Mouse	1:1000
	ATPB	Abcam	Ab14730	Mouse	1:1000

Table 1: List of primary antibodies

Note

Primary antibodies raised in different species are combined for co-staining, as follows:

- Mouse anti-LAMP1 and Rabbit anti-TMEM192
- Mouse anti-ATPB and Rabbit anti-TMEM192

10 Incubate cells at Room temperature with diluted primary antibodies for 01:00:00 .

1h

Note

This should be done in a humid chamber to avoid samples drying out. Cover a glass plate with parafilm and add 20 µL of primary antibody dilution to the relevant labelled area on the parafilm. Using tweezers, place each coverslip on the primary antibody solution (facing downward, so the cells are in contact with the antibody).

11 Wash the coverslips 3 times with 0.2% (w/v) BSA in PBS + 0.02% sodium azide. (00:05:00 per wash).

5m

12 Prepare a combination of Secondary antibodies as described below (see Table 2 for more information about the secondary antibodies). Antibodies are diluted in PBS +0.2%BSA+0.02% sodium azide.

- anti-Mouse Alexa 488 (1:500) and anti-Rabbit Alexa 594 (1:500).

	A	B	C	D	E
	Antibody	**Conjugated Fluorophore**	**Company**	**Cat. number**	**Host Species**
	anti-Mouse	Alexa 488	Invitrogen	A21206	Donkey
	anti-Rabbit	Alexa 594	Invitrogen	A11012	Goat

Table 2: List of fluorophore-conjugated secondary antibodies

13 Add 0.5 µL Hoechst 33342 solution for nuclear staining.

14 Incubate cells at Room temperature with diluted secondary antibodies for 01:00:00. Do this in a humid chamber on a piece of Parafilm. Put a 60 µL drop of diluted antibodies on the parafilm. Carefully place coverslip on the droplet, with the side containing attached cells, facing inward, making contact with the droplet. 1h

15 Wash cells, 3 times, with 3 mL PBS +0.2%BSA+0.02% sodium azide.

16 Rinse cells by dipping briefly in MilliQ water and gently dry on Kleenex wipes.

17 Label microscope glass slides (preferably the one with frosted side) according to the primary antibody used. Take note of the emission wavelength of the probe on the secondary antibodies.

18 Add a drop of VECTASHIELD antifading Mounting media.

19 Mount cover slip (containing cells) on the glass slide, ensuring that the side containing the cells is facing inward, making contact with the oil. Allow to dry for 00:30:00, ensuring slides are prevented from direct light. 30m

20 Store slides at 4 °C or view immediately on a confocal microscope.

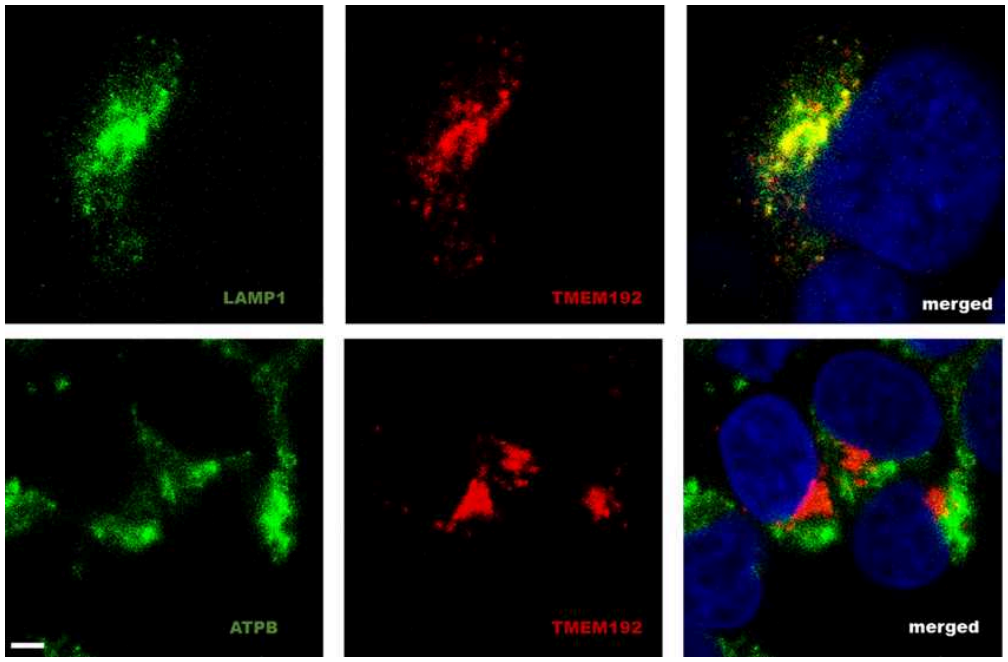

Figure 1: Immunofluorescence images of HEK293 cells showing localisation of endogenous TMEM192 with LAMP1 (a lysosomal marker) and ATPB1 (a mitochondrial marker). Scale bar is 2 µm.