

Immunofluorescence staining (cells)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- > 1X PBS (Sigma, D-8537)
- > 4% paraformaldehyde made fresh (Electron Microscopy Sciences, RT15710)
- > Triton-X100 (Sigma, X100-100ML)
- > Aurion BSA (Aurion, 900022)
- > Donkey Serum (Sigma, S30-100ML)
- > Goat Serum (Sigma, S26-100ML)
- > Tween 20 (Sigma, P2287-500ml)
- > Fluorescence mounting media with DAPI (abcam, ab104139)
- >

Procedure

1. Aspirate medium, wash cells seeded in 8-well chamber slide. When they reach 50-70% coverage, wash quickly with 500 ul of 1X PBS, for 3 times.
2. Fix the cells with 4% paraformaldehyde made fresh in 1X PBS for 15 minutes.
3. Wash with 1X PBS 3 times for 5 minutes each.
4. Permeabilize the cells with 0.2% to 0.3% Triton X-100 made in 1X PBS for 15 minutes.
5. Wash 3 times with 1X PBS for 5 minutes each.
6. Blocking and primary antibody incubation as following:

Primary ABs								
	A	B	C	D	E	F	G	H
1	Antibody	Reactivity	Host	RRID	Company	#Cat	Blocking in donky serum and Aurion BSA	Dilution in donkey serum and Aurion BSA
2	SMPD1	H and M	R	AB_3086745	LSBio	LS-C334919	10% and 5% 1h R.T.	1:500 in 5% and 3% 1h R.T.
3	Ceramide	All	M	AB_259087	Sigma	C8104-50TST	(goat serum) 10% and 0.5% 1h R.T.	1:20 in (goat serum) 5% and 0.5% o.n. at 4°C
4	Vimentin	H and M	G	AB_355153	R&D Systems	AF2105	10% and 1% 1h R.T.	25µg/ml in 5% and 0.5% o.n. at 4°C
5	E-Cadherin	H and M	R	AB_2291471	cell signaling	3195S	10% and 1% 1h R.T.	1:100 in 5% and 0.5% o.n. at 4°C
6	Ras (G12D Mutant Specific)	H and M	R	AB_2728748	cell signaling	14429S	10% and 1% 1h R.T.	1:20 in 5% and 0.5% o.n. at 4°C
7	Phosphatidylserine	all	M		Merk	ZMS1038	10% and 1% 1h R.T.	1:500 in 5% and 0.5% o.n. at 4°C

7. Wash the cells with 0.1% Tween 20 in 1X PBS 3 times for 10 minutes each.

8. Apply an appropriate fluorophore-conjugated secondary antibody in in 5% (donkey/goat serum) and 0.5% (Aurion BSA) 1h at 1:250:

Secondary ABs								
	A	B	C	D	E	F	G	H
1	Antibody	Reactivity	Labeling	Company	#Cat			
2	Donkey anti-rabbit (FITC)	rabbit	FITC	Jackson I. R	711-095-152			
3	Donkey anti-rabbit (Cy3)	rabbit	Cy3	Jackson I. R	711-165-152			
4	Donkey anti-rabbit (Cy5)	rabbit	Cy5	Jackson I. R	711-175-152			
5	Donkey anti-goat (Cy5)	goat	Cy5	Jackson I. R	705-175-147			
6	Goat anti-Mouse IgM (Heavy chain) Alexa Fluor™ 488	mouse	Alexa488	Thermo Fischer Scientific	A-21042			
7	Donkey anti-mouse (Cy5)	mouse	Cy5	Jackson I. R	715-175-151			

9. Wash the cells with 0.1% Tween 20 in 1XPBS 3 times for 10 minutes each.

10. Mount the coverslip using Fluorescence mounting media with DAPI.

11. image using zeiss microscope.