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Intracellular MAP2 staining for flow cytometry

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocols describes methods for intracellular staining of MAP2 for flow cytometry.

Materials

1. Warm NDM, trypsin, FBS
2. Take eBioscience ICC Fix and 10X perm solution out at RT for at least one hour before using
3. Make ~8 mL of 1X perm per tube of cells using molecular bio grade water per sample
 - Also make 10 more mls for block buffer (9mL water + 1mL 10x perm)
4. Block buffer (10ml, make in 1x Perm buffer)
 - 0.2M Glycine (0.1504g)
 - 2.5% FBS (0.25mL)
 - 9.75ml Perm buffer
5. In general, minimize the number of tubes and tips you use, and rinse everything as much as possible to maximize cell yield

	A	B	C	D
	NDM (Guo 2014)	Unit	100 mL	
	DMEM/F12 (GIBCO)	mL	97.5	
	0.5% FBS (GIBCO)	mL	0.5	
	3.5 mM glucose (Sigma)*	mg	63.056	
	penicillin/str eptomycin (GIBCO)	mL	1	
	N2 supplement (GIBCO)	mL	1	
	Filter sterilize after combining			

Troubleshooting



Prepare cells

- 1 Add 100ul FBS to eppendorf tubes to be used for collecting cells
- 2 Trypsinize cells (6wp) with 600ul 0.025% trypsin
- 3 Incubate 5 minutes
 - 3.1 Gently pipette trypsin to dislodge cells
 - 3.2 add to FBS
- 4 Incubate plate an extra minute at 37C
- 5 Rinse cells into tube with 600ul NDM, making sure all cells are recovered. Gently singularize
- 6 Spin 1000rpm 5min
- 7 Resuspend in 1ml PBS
- 8 Spin 1000rpm 5min

Fix, Permeabilize, Stain

- 9 Aspirate supernatant
 - 9.1 add 50ul fix buffer to cell pellet and mix by pipetting



- 10 Incubate **in dark** at RT for 20m on rocker (cover with foil)
- 10.1 *If needing to store: Do 2 PBS rinses, spin down 600xG after each. Store in PBS in dark at 4C*
- 11 Add 1 ml permeabilization buffer
- 11.1 Pellet cells, spin 1000rpm 5min
- 12 Resuspend in 1 ml perm Buffer and count cells
- 12.1 Pellet cells, spin 1000rpm 5min
- 13 Resuspend cells in 50 ul block buffer and allow to block 10 min at room temp on rocker
- 14 Add 50ul block buffer containing proper amount of antibody
- 14.1 *For MAP2 staining: use 1ul a-MAP2-488 antibody per 200,000 cells (ab225316 aka Abcam EPR19691 – 488 Conjugated)*
- 15 Incubate for 30min at RT on rocker covered in foil. Rock at very slow speed
- 16 Add 1ml 1x perm to cell / ab suspension
- 16.1 spin 600g 6 min and aspirate supernatant
- 17 Rinse with 1 mL 1x perm



17.1 spin 600g for 6 min

18 Resuspend in 100ul FACS buffer

19 If there are any doubts about clumping, filter through 40 um filter, and sort cells