ADVANCED BIOIMAGING FACILITY

Fluorescence Labeling

Fluorescence Staining:

1. Rinse the cells three times with 1 mL of phosphate buffered saline without calcium or magnesium (PBS^-).
2. Aspirate the PBS^- solution off of the coverslips.
3. Apply 1 mL of 4% paraformaldehyde (PFA) in PBS^- to each sample and leave for 10-15 minutes at 23°C.
4. Wash the cells three times with 1 mL of PBS^-.
5. Permeabilize the cells with 1 mL of 0.2% Triton-X-100 detergent in PBS^- for 3 minutes.
6. Wash the cells three times with 1 mL of PBS^-.
7. Block non-specific antibody binding for 10-15 minutes with 1 mL* of 5% bovine serum albumin (BSA) in PBS^-. For stronger blocking goat serum can be used.
8. Make sure to aspirate the coverslip well and to dry the area around the coverslip.
9. Apply 100 µL of 1° antibody solution (diluted in PBS^- containing 2% BSA) for one hour at 23°C or overnight at 4°C.
10. Wash the cells three times with 1 mL of PBS^-.
11. Apply 100 µL of 2° antibody solution (diluted in PBS^- containing 2% BSA) for one hour at 23°C. **Note:** Do not leave the 2° antibody on overnight. It will not improve specific labeling and will increase non-specific binding.
12. Wash the cells three times with 1 mL of PBS^-.
13. Apply 100 µL of DAPI (5 mg/ml stock, 1:5000 dilution to 1 µg/ml) and/or phalloidin-AF555 solution (diluted 1:1000 in PBS^- with 2%BSA) for 15 minutes at 23°C. **Note:** DAPI/phalloidin can be added together and can be combined with the 2° antibody step.
14. Wash the cells three times with 1 mL* of PBS^- and they are ready to mount.

Mounting the Coverslips:

1. Clean microscope slides with a Kimwipe™ moistened with 70% ethanol (EtOH).
2. Label the microscope slide for each sample.
3. Use the wooden end of a cotton swab handle to place a small drop of cytoseal 60 in the centre of the microscope slide.
4. Remove as much liquid as possible from the coverslip. Lift the corner of the coverslip with a needle tip and carefully grab a corner using fine tipped tweezers.
5. Tilt the slide and dry any excess liquid at the corner of the coverslip on a Kimwipe™.
6. Invert the slide and place it gently at a 45^{o} angle onto the drop of mounting medium.
7. Use the cotton end of a cotton tip applicator to gently press down on the coverslip and displace any air bubbles in the mounting media to the edges of the coverslip.
8. Leave the slides covered with foil overnight so the mounting medium can cure.
   Note: Do not use an airtight cover for the samples or the mounting medium will not cure.

Live cell labeling with nuclear and mitochondrial dyes.

1. Remove the DMEM medium from the cells.
2. Apply 1 mL of DMEM containing MitoTracker RedCMX-Ros (Make a 1 mM stock and use at 2-100 nM) and Hoechst 33342 (Use a 1:400 dilution of 1 mg/mL stock).
3. Place the cells at 37^{o}C for 10 minutes.
4. Rinse once with 1 mL of DMEM culture media.
5. Place in 1 mL of fresh culture media and take to the microscope.

ADVANCED BIOIMAGING FACILITY

Fluorescent Labeling Reagents

Product	Company	Catalogue #	Notes
DMEM	Invitrogen	11885-084	low glucose, L-glutamine, 110 mg/ml sodium pyruvate, pyridoxine hydrochloride
DMEM phenol red free	Invitrogen	11054-020	Same as above, but no phenol red and you need to add L-glutamine.
Non-essential amino acids	Invitrogen	11140-050	Add 5 mL of stock to DMEM
Penicillin-Streptomycin (Pen/Strep)	Invitrogen	15140-122	Add 5 mL of stock to DMEM
L-Glutamine	Invitrogen	25030-081	Add 5 mL of stock to DMEM
Fetal Bovine Serum (FBS)	Invitrogen	26140-079	Add 50 mL to DMEM
Geneticin (G418)	Invitrogen	11811-031	Add to DMEM for stable GFP expressing cells. 2.5 mL of 100 mg/mL stock/bottle for 0.5 mg/mL.
PBS^{-}	Invitrogen	70011-044	10X solution ph 7.4
Paraformaldehyde (PFA)	Polysciences	50-00-0	Specify 16%, dilute 1:4 for 4%. Always work under Fume Hood.
Coverslips	Fisher	12-544A	Any form but thickness #1.5
Triton-X-100	Fisher	BP151-100	Very viscous, use % v/v
Bovine serum albumin (BSA)	Jackson Immuno Research	001-000-162	IgG-free, Protease-free. Drop on top of liquid (PBS), dissolve by gravity. Avoid shaking. Use % w/v.
Phalloidin-Alexa555	Invitrogen	A34055	Use at a 1:1000 dilution.
DAPI (dilactate)	Invitrogen	D3571	5 mg/ml stock, 1:5000 dilution(1 µg/ml) Can be added during 2^{o} Antibody or phalloidin labelling steps.
1^{o} Mouse monoclonal anti-alpha tubulin	Sigma	T9026	Use at a 1:200 dilution.
Cytoseal 60	Fisher	23-244-256	To get a nice small drop use the end of a wooden cotton tip applicator.
Tubulin 2^{o} antibody AlexaFlour546 goat anti-mouse	Invitrogen	A11003	1:2000 dilution of molecular probes stock
MitoTracker Red CMX-Ros	Invitrogen	M7512	Make a 1 mM stock. Use at 2-100 nM.
Hoechst 33342	Sigma-Aldrich	14533	2.5 µg/mL 1:400 dilution of 1 mg/mL stock

Updated: 8 April 2014

ABIF

ADVANCED BIOIMAGING FACILITY

Updated: 8 April 2014