

ModDetect™ IF Protocol

I. Reagents Required

Product	Preparation	Suggested Product(s)
Phosphate Buffered Saline (PBS)	Use 10X PBS, pH 7.2 (0.2 M Potassium Phosphate, 1.5 NaCl) Dilute appropriate volume to 1X with deionized water.	MB-008
Formaldehyde Fixative	Dilute to 1% in PBS buffer. Use high-quality formaldehyde.	KHFO01
Permeabilization Buffer	Prepare an appropriate volume of 0.2% (v/v) Triton X-100 in PBS buffer.	
Antibody Dilution Buffer	Prepare 100 mL of PBS Wash Buffer supplemented with 1 mL of normal serum of the same species as the host used for the secondary antibody.	See Normal Serums
Fluorescent Secondary Reporter	Prepare dilution of fluorescent-conjugated secondary antibody (1:200) or fluorescent-conjugated streptavidin (1:200) in Antibody Dilution Buffer. Use secondary antibody conjugate against the same species as the primary antibody.	See Secondary Antibodies
Bis-Benzimide Solution	Prepare reagent at 0.5 mg/mL in PBS buffer.	
Aqueous Mounting Media		KHH001

II. Procedure

1. Grow cells as expected. Remove culture medium.
2. Gently wash cells 3 times with ice-cold PBS for 5 minutes per wash.
3. Fix cells by adding a volume of 4% paraformaldehyde in PBS equal to the original volume of culture medium and block with 5% non-host serum in 0.1% PBST. Incubate on ice for 30 minutes. Remove the fixative and wash as in Step 2.
4. Permeabilize cells for 20 minutes on ice with permeabilization buffer.
5. Prepare primary antibody with recommended dilution of 1:200-1:2,000 in antibody dilution buffer. React cells with primary antibody for 1 hour at room temperature.
6. Wash as in Step 2.
7. Prepare dilution of fluorescent-conjugated affinity-purified secondary antibody, react cells for 1 hour at room temperature with reagent. Alternatively, add fluorescent-conjugated streptavidin diluted 1:200 in PBS buffer. React for 30 minutes at room temperature.
8. Wash as in Step 2.
9. Counterstain cells with bis-benzimide solution for 15 minutes at room temperature.
10. Wash as in Step 2.
11. Add aqueous mounting agent. Affix coverslips to slides. Allow coverslips to dry in the dark before viewing.

III. Supporting Products

Product	Item No.
FACS and IF Staining Buffer with Goat Serum	MB-088-0500
FACS and IF Staining Buffer with BSA	MB-089-0500
FACS and IF Staining Buffer with FBS	MB-086-0500
Mouse IgG (gamma 1, 2a, 2b and 3 chain) DyLight™ 488 Conjugated	610-441-C46
Mouse IgG (gamma 1, 2a, 2b and 3 chain) DyLight™ 680 Conjugated	610-444-C46
Mouse IgG (gamma 1, 2a, 2b and 3 chain) DyLight™ 800 Conjugated	610-445-C46

Product	Item No.
Mouse IgG (gamma 1, 2a, 2b and 3 chain) ATTO 425 Conjugated	610-451-C46
Mouse IgG (gamma 1, 2a, 2b and 3 chain) ATTO 532 Conjugated	610-453-C46
Mouse IgG (gamma 1, 2a, 2b and 3 chain) ATTO 550 Conjugated	610-454-C46
Mouse IgG (gamma 1, 2a, 2b and 3 chain) ATTO 647N Conjugated	610-456-C46
Mouse IgG (gamma 1, 2a, 2b and 3 chain) ATTO 655 Conjugated	610-457-C46

Supporting Products Available via antibodies-online

Endosome Marker Antibodies	Item No.
Anti-EEA1 (RABBIT) Antibody	ABIN521882
Anti-RAB7B (AA 100-199) Antibody	ABIN567027
Anti-RAB5 Antibody	ABIN361846
Anti-RAB5B (C-Term) Antibody	ABIN1439995
Anti-RAB5C (C-Term) Antibody	ABIN6254194
Anti-RAB7A (C-Term) Antibody	ABIN720191
Anti-RAB9A (RABBIT) Antibody	ABIN564041

Lysosome Marker Antibodies	Item No.
Anti-Cathepsin L (AA114-288) Antibody	ABIN7431587
Anti-CTSA (N-Term) Antibody	ABIN654433
Anti-Cathepsin D (C-Term) Antibody	ABIN6254162
Anti-LAMP1 (AA 80-280) Antibody	ABIN3016286
Anti-LAMP2 (AA 191-362) Antibody	ABIN7437248
Anti-Cathepsin K (AA 54-317) Antibody	ABIN7441856
Anti-Cathepsin S (AA 115-331) Antibody	ABIN7434368

Cytoskeleton Marker Antibodies	Item No.
Anti-Vimentin (AA 371-466) Antibody	ABIN672786
Anti-Alpha Tubulin Antibody	ABIN93891
Anti-TUBB Antibody	ABIN93914
Anti-Smooth Muscle Actin (N-Term) Antibody	ABIN6254917
Anti-ACTC1 Antibody	ABIN2855212
Anti-ACTN2 Antibody	ABIN2855582
Anti-ACTG2 (AA 3-376) Antibody	ABIN7440569
Anti-Myosin (AA 1069-1331) Antibody	ABIN7439273
Anti-Alpha Actinin Antibody	ABIN7073023
Anti-Dystrophin (AA 346-635) Antibody	ABIN1679546
Anti-ACTN3 (N-Term) Antibody	ABIN6258681
Anti-Cytokeratin 8/18 Antibody	ABIN285686