**ImageStream® Endosome/Lysosome Direct Protocol**

**Probing for Internalization and Co-localization to Endosomes & Lysosomes**

**Samples: (2 x $10^6$ cells per test)**

Single fluorescent color control samples – unstained, CD71 or CD107a FITC, etc

Experimental samples – untreated, positive control treatment, experimental treatment

**Materials:**

1. Antibody to test protein (labeled or unlabeled)
2. FITC anti-CD71 (BD Pharmingen cat#555536 or Invitrogen/Caltag cat#MHCD7101 for FITC anti-human, #RM5301 for FITC anti-mouse)
3. FITC- or Spectral Red anti-CD107a (Lamp-1) (Southern Biotech cat#9835-13 for Spectral Red anti-human - reacts with human and mouse; cat#1920-04 for FITC anti-mouse, #9835-01 or BD Pharmingen cat#555800 for FITC anti-human)
4. Fix buffer (PBS / 1% Formaldehyde)
5. Wash buffer (PBS / 2% FBS)
6. Perm/Wash buffer (PBS / 2% FBS / 5% Saponin)
7. Siliconized polypropylene tubes: Sigma (Cat. T4691 for 0.6mL and Cat. T4816 for 1.5mL)

**Cell preparation**

We used Ramos cells cultured in RPMI supplemented with 10% fetal calf serum in an incubator containing 5% $CO_2$ at 37° C.

**Staining protocol**

Staining done in siliconized polypropylene (NOT POLYSTYRENE) microcentrifuge tubes. All washes done at 300 x g 10' 4° C in a swinging bucket rotor.

1. Culture cells to mid-exponential growth or isolate primary cells according to procedure.
2. Incubate with labeled or unlabeled test protein 30' on ice (negative control) or various time points @ 37° C.
3. Wash with Wash buffer, then stain cells with surface markers in 100μL Wash buffer for 20' 4° C.
4. Wash with Wash buffer, then fix with 100μL of Fix buffer for 20' RT.
5. Wash cells with 1mL of Perm/Wash, then stain cells with 10μg/mL anti-CD71 and/or anti-CD107and/or secondary to the unlabeled test protein in Perm/Wash for 30' RT.
6. Wash cells with 1mL of Perm/Wash buffer and resuspend cells in 50μL Fix buffer.