Immunostaining Protocol

1. Prepare the cells
   - HeLa cells were transfected with membrane-targeted or cytosolic MT1-MMP biosensor.
2. Wash cells twice with PBS.
3. Fixation, staining with primary antibody, permeabilization and blocking

Primary antibody -----> fixation -----> blocking

A) Chilled cells were incubated with GFP antibody (1:200 in CO2-independent medium without serum) at 4 C for 60 min.

B) Wash three times with ice-cold PBS to remove unbound antibody; Fix cells with 4% paraformaldehyde at RT for 20 min.

C) Wash three times with PBS.

D) Blocking with 10% BSA for 30 min.

Fixation -----> Primary antibody -----> blocking

A) Fix cells with 4% paraformaldehyde at RT for 10 min.

B) Wash three times with PBS; incubated with GFP antibody (1:200 in CO2-independent medium without serum) at 4 C for 60 min.

C) Wash three times with ice-cold PBS to remove unbound antibody

D) Blocking with 10% BSA for 30 min.

Fixation -----> permeabilization -----> Primary antibody ----> blocking

A) Fix cells with 4% paraformaldehyde at RT for 10 min.

B) Wash three times with PBS; permeabilize samples with 0.1% (v/v) Triton X-100 at RT for 20 min

C) Wash three times with PBS; blocking with 10% BSA for 30 min.

D) Incubated with GFP antibody (1:200 in CO2-independent medium without serum) at 4 C for 60 min.

E) Wash three times with ice-cold PBS to remove unbound antibody.

1. Stained with fluorescence-conjugated secondary antibody (1:100) in 1% BSA-PBS at RM for 30-60 min.
2. Remove secondary antibody; wash three times with PBS.
3. Get ready mounting solution. Mount samples with antifade solution.
4. Taking imaging under microscope.