Labeling reaction using CLIP-Cell TMR

Overview

Protocol

1. Dilute the labeling stock solution 1:200 in medium to yield a labeling medium of 5 µM dye substrate. Mix dye with medium thoroughly by pipetting up and down 10 times (necessary for clean backgrounds). For best performance, add the CLIP-Tag substrate to complete medium, including serum (0.5% BSA can be used for experiments carried out in serum-free media). Do not prepare more medium with CLIP-Tag substrate than you will consume within one hour.
2. Replace the medium on the cells expressing a CLIP-Tag fusion protein with the CLIP-Tag labeling medium and incubate for 30 to 60 minutes.
3. Wash the cells with three changes of tissue culture medium with serum for 15 to 30 minutes. Replace the medium one more time to remove non-reacted CLIP-Tag substrate that has leaked out of the cells.
4. Image the cells using an appropriate filter set. CLIP-Tag fusion proteins labeled with CT-TMR should have an excitation maximum at 554 nm and an emission maximum at 580 nm, and can be imaged with standard rhodamine filter sets.

   We recommend routinely labeling one well of non-transfected or mock-transfected cells for comparison.