Labeling reaction using SNAP-Cell TMR-Star

Overview

Protocol

1. Dilute the labeling stock solution 1:200 in medium to yield a labeling medium of 2 µM dye substrate. Mix dye with medium thoroughly by pipetting up and down 10 times (necessary for clean backgrounds). For best performance, add the SNAP-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 SNAP-Tag substrate than you will consume within one hour.
2. Replace the medium on the cells expressing a SNAP-Tag fusion protein with the SNAP-Tag labeling medium and incubate for 30 minutes.
3. Wash the cells twice with tissue culture medium with serum and incubate in fresh medium (without label) for 30 minutes. Replace the medium one more time to remove unreacted SNAP-Tag substrate that has diffused out of the cells.
4. Image the cells using an appropriate filter set. SNAP-Tag fusion proteins labeled with TMR-Star 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.