Labeling reaction using BG-Alexa Fluor® 647 on the surface of living cells

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 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 with an appropriate filter set. SNAP-Tag fusion proteins labeled with BG-Alexa Fluor® 647 should have an excitation maximum at 652 nm and an emission maximum at 670 nm, and can be imaged with standard 635 nm or 650 nm diode laser excitation.

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