invitrogen™

Labeling of cellular targets with Qdot® anti-Fluorescein Conjugates

Quantum Dot
invitrogen nanocrystal technologies

Molecular Probes™
invitrogen detection technologies

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Labeling of cellular targets with Qdot® anti-Fluorescein Conjugates

Materials

- Adherent cell lines and culture mediums.
- Glass chamber tissue culture slides.
- Phosphate-buffered saline (PBS).
- Fixatives (such as methanol, acetone or paraformaldehyde)
- Bovine serum albumin (such as IgG-free BSA from Jackson ImmunoResearch Laboratories).
- Fluorescein-conjugated primary or secondary antibody
- Qdot® anti-Fluorescein Conjugate (1 μM stock solution)
- Polyvinyl alcohol-based mounting medium (such as Polyvinyl Alcohol Mounting Medium with DABCO from Sigma).

Notes:

1. Centrifuge at 5,000-10,000 x g, for 5-10 min. reserving the supernate, prior to using the material.
2. Some PAP pens can quench the signal from the quantum dots. If your protocol requires the use of a PAP pen, we recommend the ImmEdge Hydrophobic Barrier Pen (H-4000) from Vector Labs.

Procedure

All steps of the procedure will be conducted at room temperature unless specified.

1. Culture cells on chamber slides.
2. Wash cells with cold PBS for 3 x 2 min.
3. Fix cells with 70% methanol/30% acetone (v/v) for 15 min at–20 ^{0}C. Methanol and acetone are stored at –20 ^{0}C separately and mixed together before use. Cells need to be permeabilized with detergent (such as 0.25% Triton X-100) if the fixative is paraformaldehyde or other cross-linking fixatives.
4. Wash cells with PBS for 3 x 2 min.
5. Block cells with PBS containing 2% BSA and 0.05% Triton X-100 for 30 min.
6. Incubate cells with the primary antibody diluted in the blocking buffer for 1 hour at 37 ^{0}C.
7. Wash cells with PBS for 3 x 5 min.
8. Incubate cells with the fluorescein conjugated secondary antibody diluted with the blocking buffer (final concentration is 1-5 μg/ml) for 30 min at 37 ^{0}C. This step is omitted if the primary antibody is directly conjugated to fluorescein.
9. Wash cells with PBS for 3 x 5 min.
10. Incubate cells with Qdot anti-Fluorescein diluted in PBS containing 2% PBS for 1 hour at 37 ^{0}C. Optimal dilution should be experimentally determined by the individual user to obtain best signal-to noise-ratio. Typically, a final concentration of 5-20 nM is satisfactory for most labeling experiments.
11. Wash cells with PBS for 3 x 5 min.
12. Mount with coverslip using a polyvinyl alcohol-based mounting medium.