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Attachment of Anti-GFP Antibodies to Microspheres for Optical Trapping Experiments

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Abstract

In vitro motility assays enabled the analysis of coupling between ATP hydrolysis and movement of myosin along actin filaments or kinesin along microtubules. Single-molecule assays using laser trapping have been used to obtain more detailed information about kinesins, myosins, and processive DNA enzymes. The combination of in vitro motility assays with laser-trap measurements has revealed detailed dynamic structural changes associated with the ATPase cycle. This protocol describes a method for attaching anti-GFP (green fluorescent protein) antibodies to microspheres. GFP-motor fusion proteins can then be adsorbed to the microspheres for use in single-molecule motility studies and optical trapping experiments.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Anti-GFP antibody (mouse monoclonal, 0.05 mg/mL in PBS [mAb3E6]; Qbiogene/MP Biomedicals or similar)

Beads (carboxylated microspheres, 0.5- to 1- μ m diameter; Polysciences or similar)

Bovine serum albumin labeled with tetramethyl rhodamine isothiocyanate (TMR-BSA)

Phosphate-buffered saline (PBS; 100 mM)

Equipment

Centrifuge

Incubator preset to 22°C

METHOD

1. Dilute 10 μ L of beads in 100 μ L of PBS. Centrifuge at 20,000g for 1 min at 22°C.

RELATED INFORMATION

A detailed discussion of optical trapping can be found in **Optical Traps to Study Properties of Molecular Motors** (Spudich et al. 2011a). A protocol is also available for **The Optical Trapping Dumbbell Assay for Nonprocessive Motors or Motors That Turn around Filaments** (Spudich et al. 2011b).

2. Resuspend beads in another 100 μ L of PBS and repeat Step 1.
3. Resuspend beads in 20 μ L of anti-GFP antibody +1 μ L of TMR-BSA. Incubate for 5 min at 22°C.
4. Centrifuge beads at 20,000g for 1 min at 22°C.
5. Resuspend beads in 20 μ L of PBS.
6. Repeat Steps 4 and 5 three times.

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