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The Optical Trapping Dumbbell Assay for Nonprocessive Motors or Motors That Turn around Filaments

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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 the preparation of biotin–actin filaments and coverslips coated with polystyrene beads. These are then used in optical trapping dumbbell assays to study interactions between motors and filaments.

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.

RECIPE: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

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

AB <R>

ABSA buffer (AB + 1 mg/mL bovine serum albumin [BSA])

ATP

Beads (polystyrene or glass, 1–2 μ m) in 0.1% Triton X-100

Biotinylated actin (50 μ M; for preparation, see Rock et al. [2000])

KCl

Motor to be studied (e.g., myosin VI)

NAV beads (1- μ m biotinylated polystyrene beads, coated with neutravidin and thoroughly washed in ABSA buffer [Rock et al. 2000])

Nitrocellulose (0.1% in isoamyl acetate)

O₂ scavengers (25 µg/mL glucose oxidase, 45 µg/mL catalase)

Store in PBS + 50% v/v glycerol at –20°C. Add 0.1% glucose to this mixture for the assay.

Tetramethylrhodamine (TMR)-phalloidin (rhodamine phalloidin; Invitrogen)

Equipment

Bath sonicator

Coverslips (18 mm²)

Spectrophotometer

Tape (double-stick)

Vacuum grease

METHOD

Preparation of Biotin–Actin Filaments

- 1 Thaw 50 µM biotinylated actin. Sonicate in a bath sonicator for 30 sec. Place for 1 h at 4°C to depolymerize any actin complexes present in the solution.
- 2 Add KCl (50 mM final) and ATP (2 mM final). Allow the actin to polymerize for 1 h at 4°C.

This results in slow polymerization of the actin into very long (many 10 µm) filaments.

- 3 Add stoichiometric (~50 µM) TMR-phalloidin prior to use.

Actin filaments can be used for up to 1 wk, stored at 4°C.

Preparation of Bead-Coated Coverslips

- 4 Sonicate 1-µm polystyrene beads in 0.1% Triton X-100 for 30 sec in a bath sonicator.
- 5 Coat the coverslips with a monolayer of the sonicated beads. Use a solution of sonicated beads with an optical density of 0.15 at 595 nm to provide a good density of surface platforms on slides.
- 6 Dip coverslips in a solution of 0.1% nitrocellulose in isoamyl acetate and dry.

Preparation of Flow Cell for Study of Motor

- 7 Make a flow cell using a beaded coverslip and double-stick tape (Rock et al. 2000).
- 8 Coat the coverslip surface with anti-GFP antibodies. Wait for 4 min.
- 9 Wash the slide with 10 µL of ABSA buffer.

- 10 Flow in the motor, at a dilution that should result in single-molecule motility. Wait another 4 min.

For myosin VI, the concentration of motor used is 1 nM, but it varies depending on the motor and the type of linkage.
- 11 Wash the slide with 10 μ L of ABSA buffer.
- 12 Flow in a mixture of O₂ scavengers, ATP (~nM–mM concentrations), 1:300 NAV beads, and 1:4000 TMR-actin in AB.
- 13 Coat the edges of the flow cell with vacuum grease to prevent evaporation during the assay. The sample is ready to image.

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 **Attachment of Anti-GFP Antibodies to Microspheres for Optical Trapping Experiments** (Spudich et al. 2011b).

RECIPE

AB
25 mM imidazole HCl at pH 7.4
25 mM KCl
5 μ M calmodulin
1 mM EGTA
10 mM dithiothreitol (DTT)
4 mM MgCl₂
2 mM ATP

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