

STAINING NONFILAMENTOUS ACTIN WITH VITAMIN-D BINDING PROTEIN

Indirect Method

Materials

1. Gc-globulin (vitamin-D binding protein; Calbiochem 345802), prepare 2 mg/ml stock in PBS solution A, clarify 25,000 rpm for 20 min in a 42.2Ti rotor.
2. PBS/BSA: PBS solution A with 1% BSA (Boehringer Mannheim 100 350) and 0.1% NaN₃, stored at 4°C.
3. Diluted Gc-globulin, dilute 2 mg/ml stock with PBS/BSA to obtain 1-10 ug/ml. Need 200 ul per coverslip.
4. Primary antibodies against Gc-globulin (DAKO A021), diluted 1:500 with PBS/BSA.
5. Secondary goat anti-rabbit antibodies, typically diluted 1:100.

Procedure

1. Fix and extract cells following the formaldehyde-acetone protocol.
2. Wash the coverslip for 10 min in PBS/BSA.
3. Cut a piece of parafilm to match the area of staining and pipet diluted Gc-globulin on the piece. Invert the coverslip onto the parafilm. Prepare a 100 mm plastic petri dish containing a piece of wet filter paper. Place 2 wooden sticks in the dish and put coverslip upside down on the sticks. Let sit at room temperature for 60 min.
4. Wash gently 3x, 10 min each with PBS/BSA on a shaker. Fill a coverslip box with PBS/BSA and sink the coverslip to the bottom. The covering parafilm should float off.
5. Stain with antibodies against Gc-globulin and secondary antibodies (see Immunofluorescence protocol).

Direct Method

Materials

1. PBS/BSA: PBS solution A with 1% BSA (Boehringer Mannheim 100 350) and 0.1% NaN₃, stored at 4°C.

2. Fluorescein-Gc-globulin. Diluted to 10 ug/ml in PBS/BSA.

Procedure

1. Fix and extract cells following the formaldehyde-acetone protocol.

2. Wash the coverslip for 10 min in PBS/BSA.

3. Cut a piece of parafilm to match the area of staining and pipet diluted fluorescein-Gc-globulin on the piece. Invert the coverslip onto the parafilm. Prepare a 100 mm plastic petri dish containing a piece of wet filter paper. Place 2 wooden sticks in the dish and put coverslip upside down on the sticks. Let sit at room temperature for 60 min.

4. Wash gently 3x, 10 min each with PBS/BSA on a shaker. Fill a coverslip box with PBS/BSA and sink the coverslip to the bottom. The covering parafilm should float off.
