Atg12 Antibody (Mouse Specific) #2011

Protocol

Immunofluorescence (Immunocytochemistry)

A. Solutions and Reagents

Achieve higher quality immunofluorescent images using the efficient and cost-effective, pre-made reagents in our #12727 (/product/productDetail.jsp?productId=12727) Immunofluorescence Application Solutions Kit

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (9808 (/product/productDetail.jsp?productId=9808)) To prepare 1L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix. Adjust pH to 8.0.
2. **Formaldehyde**: 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh and store opened vials at 4°C in dark, dilute in 1X PBS for use.
3. **Blocking Buffer** (1X PBS / 5% normal serum / 0.3% Triton™ X-100): To prepare 10 ml, add 0.5 ml normal serum from the same species as the secondary antibody (e.g., Normal Goat Serum (#5425 (/product/productDetail.jsp?productId=5425))) and 0.5 mL 20X PBS to 9.0 mL dH_2O, mix well. While stirring, add 30 µl Triton™ X-100.
4. **Antibody Dilution Buffer** (1X PBS / 1% BSA / 0.3% Triton X-100): To prepare 10 ml, add 30 µl Triton™ X-100 to 10 ml 1X PBS. Mix well then add 0.1 g BSA (9998 (/product/productDetail.jsp?productId=9998)), mix.
5. **Recommended Fluorochrome-conjugated Anti-Rabbit secondary antibodies:**
    - Anti-Rabbit IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 (/product/productDetail.jsp?productId=4412)
    - Anti-Rabbit IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 555 Conjugate) #4413 (/product/productDetail.jsp?productId=4413)
    - Anti-Rabbit IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 594 Conjugate) #8889 (/product/productDetail.jsp?productId=8889)
    - Anti-Rabbit IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 647 Conjugate) #4414 (/product/productDetail.jsp?productId=4414)
6. **Prolong® Gold AntiFade Reagent** (#9071 (/product/productDetail.jsp?productId=9071)), **Prolong® Gold AntiFade Reagent with DAPI** (#8961 (/product/productDetail.jsp?productId=8961)).

B. Specimen Preparation - Cultured Cell Lines (IF-IC)

NOTE: Cells should be grown, treated, fixed and stained directly in multi-well plates, chamber slides or on coverslips.

1. Aspirate liquid, then cover cells to a depth of 2–3 mm with 4% formaldehyde diluted in 1X PBS.

   NOTE: Formaldehyde is toxic, use only in a fume hood.

2. Allow cells to fix for 15 min at room temperature.
3. Aspirate fixative, rinse three times in 1X PBS for 5 min each.
4. Proceed with Immunostaining (Section C).

C. Immunostaining

NOTE: All subsequent incubations should be carried out at room temperature unless otherwise noted in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

1. Block specimen in Blocking Buffer for 60 min.
2. While blocking, prepare primary antibody by diluting as indicated on datasheet in Antibody Dilution Buffer.
3. Aspirate blocking solution, apply diluted primary antibody.
4. Incubate overnight at 4°C.

5. Rinse three times in 1X PBS for 5 min each.
6. Incubate specimen in fluorochrome-conjugated secondary antibody diluted in Antibody Dilution Buffer for 1–2 hr at room temperature in the dark.
7. Rinse three times in 1X PBS for 5 min each.
8. Coverslip slides with Prolong® Gold Antifade Reagent (#9071 (/product/productDetail.jsp?productId=9071)) or Prolong® Gold Antifade Reagent with DAPI (#8961 (/product/productDetail.jsp?productId=8961)).
9. For best results, allow mountant to cure overnight at room temperature. For long-term storage, store slides flat at 4°C protected from light.

posted November 2006 • revised November 2013