#12727 Store at RT and 4°C

Immunofluorescence Application Solutions Kit

1 Kit (100 tests)

Support: +1-978-867-2388 (U.S.)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Applications
IF-IC, IF-F

Species Cross-Reactivity
All

Products Included	Product #	Volume	Storage Temp
10X Wash Buffer, Phosphate Buffered Saline (PBS)	12528	35 ml	Room Temp.
16% Formaldehyde, Methanol-Free	12606	10 ml	Room Temp.
Immunofluorescence Blocking Buffer	12411	10 ml	4°C
Immunofluorescence Antibody Dilution Buffer	12378	20 ml	4°C

Description: The Immunofluorescence Application Solutions Kit is designed to conveniently provide the major supporting reagents needed for immunofluorescence in cell cultures (IF-IC) or frozen samples (IF-F). The reagents in this kit are thoroughly validated using our IF-approved antibodies* and will perform optimally with the kit's recommended protocol, ensuring accurate and reproducible results. This kit includes sufficient reagents for 100 assays based on a 100 µl assay volume.

***IMPORTANT:**
Please refer to the antibody data sheet to determine if it is validated and approved for use on cultured cell lines (IF-IC) or frozen tissue sections (IF-F) and for information regarding appropriate antibody dilution.

Some primary antibodies may require methanol fixation or permeabilzation, which will be noted on the datasheet. Methanol is not included in this kit.

Storage: All components in this kit are stable for at least 12 months when stored at the recommended temperature and left unused. Formaldehyde fixative should be used within one month once opened.

Upon receipt, #12528 and #12606 should be stored at room temperature. #12411 and #12378 should be stored at 4°C.

Reagents not supplied:

1. Unconjugated or conjugated primary antibody.
2. Fluorochrome-conjugated secondary antibody (if applicable).

NOTE: When using any primary or fluorochrome-conjugated secondary antibody for the first time, titrate the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

3. Methanol (if necessary).
4. Antifade mounting reagent, such as ProLong® Gold Antifade Reagent (#9071), with DAPI (#8961).

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

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Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity: H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#12727

Immunofluorescence Application Solutions Kit Protocol

NOTE: Please refer to the antibody data sheet to determine if it is validated and approved for use on cultured cell lines (IF-IC) or frozen tissue sections (IF-F) and for information regarding appropriate antibody dilution.

Some primary antibodies may require methanol fixation or permeabilization, which will be noted on the data sheet. Methanol is not included in this kit.

A Solutions and Reagents

NOTE: The #12528, #12411, and #12378 buffers in this kit contain 0.05% sodium azide as a preservative. Peroxidase-based fluorescence assays may be negatively affected by sodium azide.

SUPPLIED REAGENTS

1. **10X Wash Buffer, Phosphate Buffered Saline (PBS):** (#12528) Dilute to 1X Wash Buffer using reverse osmosis deionized (RODI) or equivalent grade water. Store at 4°C and use within one month once diluted.
2. **16% Formaldehyde, Methanol-Free:** (#12606) Use fresh, dilute to 4% with 1X Wash Buffer. Leftover diluted material should be discarded.
   NOTE: The screw cap allows for the entire vial contents to be used at once. To extend the product's shelf-life, small volumes should be extracted by piercing the silicone top with a needle and syringe. Store protected from light and use within one month after opening.
3. **Immunofluorescence Blocking Buffer:** (#12411) Contains goat serum as a protein blocker mixed with a mild detergent. Use as supplied and store at 4°C.
4. **Immunofluorescence Antibody Dilution Buffer:** (#12378) Use as supplied and store at 4°C.

ADDITIONAL REAGENTS (NOT SUPPLIED)

1. **Unconjugated or conjugated primary antibody**.
2. **Fluorochrome-conjugated secondary antibody** (if applicable).
   NOTE: When using any primary or fluorochrome-conjugated secondary antibody for the first time, titrate the antibody to determine which dilution provides the strongest specific signal with the least background for your sample.
3. Methanol (if necessary).
4. Antifade mounting reagent, such as **ProLong® Gold Antifade Reagent (#9071)** or **ProLong® Gold Antifade Reagent** with **DAPI (#8961)**.

B Specimen Preparation

1. **Cultured Cell Lines (IF-IC)**
   NOTE: Cells should be cultured, treated, fixed, and stained directly in multi-well plates, chamber slides or on coverslips.
   a. Aspirate liquid and cover cells to a depth of 2–3 mm with 4% formaldehyde diluted in 1X Wash Buffer.
      NOTE: Formaldehyde is toxic, use only in fume hood.
   IMPORTANT: If noted on the primary antibody data sheet, use ice-cold 100% methanol in lieu of formaldehyde as the fixative.
   b. Allow cells to fix for 15 min at room temperature.
   c. Aspirate fixative, rinse three times with 1X Wash Buffer for 5 min each.
   d. Proceed with Immunostaining (Section C).
2. **Frozen/Cryostat Sections (IF-F)**
   a. For fixed frozen tissue proceed with Immunostaining (Section C).
   b. For fresh, unfixed frozen tissue, please fix immediately, as follows:
      1) Cover sections with 4% formaldehyde diluted in 1X Wash Buffer.
      2) Allow sections to fix for 15 min at room temperature.
      3) Rinse slides three times with Wash Buffer for 5 min each.
      4) Proceed with Immunostaining (Section C).

C Immunostaining

NOTE: Unless otherwise noted, all subsequent incubations should be carried out at room temperature in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading. Do not allow slides to dry at any time during this process.

1. Methanol Permeabilization Step (if applicable): Aspirate wash buffer and cover specimen to a depth of 3-5 mm with ice-cold 100% methanol. Incubate for 10 min at -20°C, and then rinse in 1X Wash Buffer for 5 min.
   NOTE: This step is only required if noted on the primary antibody data sheet.
2. Block specimen in Blocking Buffer for 60 min at room temperature.
3. While blocking, prepare primary antibody by diluting as indicated on data sheet in Antibody Dilution Buffer.
4. Aspirate Blocking Buffer, apply diluted primary antibody.
5. Incubate overnight at 4°C.
6. Rinse three times with 1X Wash Buffer for 5 min each.
   NOTE: If using a fluorochrome-conjugated primary antibody, such as an Alexa Fluor® fluorochrome antibody, then skip to (Section C, Step 9).
7. Incubate specimen in fluorochrome-conjugated secondary antibody diluted in Antibody Dilution Buffer for 1 hr at room temperature in the dark.
8. Rinse three times with 1X Wash Buffer for 5 min each.
9. Coverslip slides with antifade reagent, such as ProLong® Gold Antifade Reagent (#9071) or ProLong® Gold Antifade Reagent with DAPI (#8961).
10. For best results, allow mountant to cure overnight at room temperature. For long-term storage, store slides flat at 4°C protected from light.

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