For Research Use Only. Not For Use In Diagnostic Procedures.

**Cellular Localization IF Antibody Sampler Kit**

1 Kit
(9 x 20 µl)

**Description:** The Cellular Localization IF Antibody Sampler Kit provides an economical means for identification of cellular organelles by fluorescence immunocytochemistry (IF-IC). This kit includes enough primary antibody to perform at least twenty IF-IC tests or two Western blots with each antibody.

**Background:** Knowledge of the subcellular location of a protein may reveal the potential role it plays in a variety of cellular processes. One can confirm the subcellular location of a marker that colocalizes with one of the organelle-specific antibodies in this kit. While these antibodies serve as powerful tools for immunofluorescence, they may also be used as western blot controls for fractionated cell lysates.

**Specificity/Sensitivity:** Each antibody in the Cellular Localization IF Antibody Sampler Kit recognizes only its specific target and does not cross-react with other family members. Each antibody has been validated for IF-IC and stains the organelles indicated above. Expression of these proteins may vary in different cells and tissues. Please see www.cellsignal.com for additional specificity/sensitivity information for individual kit components.

**Source/Purification:** Rabbit monoclonal antibodies are prepared by immunizing animals with a synthetic peptide corresponding to: the amino terminus of human β-tubulin, the sequence of human calnexin, residues surrounding Lys29 of human COX IV, the carboxy-terminal sequence of human histone H3 and human histone H2A, residues surrounding Thr298 of human fibrillarin, and residues near the amino terminus of LC3B. Mouse monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly190 of human Rab5A protein.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at −20°C. Do not aliquot the antibodies.

**Species Cross-Reactivity Key:**
- H — human
- M — mouse
- R — rat
- Hm — hamster
- Mk — monkey
- Mi — mink
- C — chicken
- Dm — D. melanogaster
- X — Xenopus
- Z — zebra fish
- B — bovine

**Applications Key:**
- W — Western
- IP — Immunoprecipitation
- IH — Immunohistochemistry
- CIP — Chromatin Immunoprecipitation
- IF — Immunofluorescence
- F — Flow cytometry
- E-P — ELISA-Peptide

**See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross reactivity and additional application protocols.**

*Product-specific protocol.*
**Immunofluorescence Protocol**

**A. Solutions and Reagents**

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

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L PBS add 50 ml 20X PBS to 950 ml dH\_2O, mix.
   **NOTE:** Adjust pH to 8.0.

2. **Formaldehyde:** 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4°C in dark.

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)) to 9 ml 1X PBS and 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 0.1g BSA (#9998), mix.

5. **Fluorochrome-conjugated Secondary Antibodies:** (Anti-mouse #4408, #4409, #8890, #4410) (Anti-rabbit #4412, #4413, #8889, #4414) (Anti-rat #4416, #4417, #4418)

Reagents specific to IF-P application:

1. **Xylene**
2. **Ethanol, anhydrous denatured, histological grade, 100% and 95%**
3. **Antigen Unmasking:**
   a. **For Citrate:** 10 mM Sodium Citrate Buffer: To prepare 1 L add 2.94 g (C\(_6\)H\(_4\)Na\(_2\)O\(_6\)H\(_2\)O) to 1 L dH\(_2\)O. Adjust pH to 6.0.
   b. **For EDTA:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA (C\(_{10}\)H\(_{16}\)O\(_{4}\)Na\(_2\)•2H\(_2\)O) to 1 L dH\(_2\)O. Adjust pH to 8.0.

**B. Specimen Preparation**

**I. 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 warm 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).

**II. Paraffin Sections (IF-P)**

**NOTE:** Do not allow slides to dry at any time during this process.

1. **Deparafrinization/Rehydration:**
   a. Wash three times in xylene for 5 min each.
   b. Wash two times in 100% ethanol for 10 min each.
   c. Wash two times in 95% ethanol for 10 min each.
   d. Rinse sections two times in dH\(_2\)O for 5 min each.

2. **Antigen Unmasking:** **NOTE:** Consult product datasheet or product webpage for specific recommendation for the unmasking solution.
   a. **For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0, then maintain at a sub-boiling temperature for 10 min. Cool slides on bench top for 30 min.
   b. **For EDTA:** Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 min at a sub-boiling temperature.
   No cooling is necessary.
3. Proceed with Immunostaining (Section C).

**III. Frozen/Cryostat Sections (IF-F)**

1. For fixed frozen tissue proceed with Immunostaining (Section C).

2. For fresh, unfixed frozen tissue, fix immediately, as follows:
   a. Cover sections with 4% formaldehyde diluted in warm 1X PBS.
   b. Allow sections to fix for 15 min at room temperature.
   c. Rinse slides three times in PBS for 5 min each.
   d. 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 fluorescent 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. Coverslip slides with Prolong™ Gold Antifade Reagent with DAPI (#8961) or Prolong™ Gold Antifade Reagent with DAPI (#6961).
7. For best results, allow mountant to cure overnight at room temperature. For long-term storage, store slides flat at 4°C protected from light.

Some CST™ antibodies work optimally using an alternate protocol. Please see product datasheet for product-specific recommendations.