

#4753 Store at -20°C

# Cellular Localization IF Antibody Sampler Kit



✓ 1 Kit  
(10 x 40 µl)

**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com  
**Web** ■ www.cellsignal.com

New 09/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Organelle	Mol. Wt.	Isotype	IF-IC Dilution
β-Tubulin (9F3) Rabbit mAb	2128	40 µl	Cytoskeleton	55 kDa	Rabbit IgG	1:200
Calnexin Antibody	2433	40 µl	Endoplasmic reticulum	90 kDa	Rabbit IgG	1:100
COX IV (3E11) Rabbit mAb	4850	40 µl	Mitochondria	17 kDa	Rabbit IgG	1:250
Rab5 Antibody	2143	40 µl	Endosomes	25 kDa	Rabbit IgG	1:100
Histone H3 Antibody	9715	40 µl	Nucleus	17 kDa	Rabbit IgG	1:50
NUP98 (C39A3) Rabbit mAb	2598	40 µl	Nuclear Envelope	98 kDa	Rabbit IgG	1:50
Fibrillarin (C13C3) Rabbit mAb	2639	40 µl	Nucleolus	37 kDa	Rabbit IgG	1:400
Atg12 Antibody (Human Specific)	2010	40 µl	Autophagosomes	16, 53 kDa	Rabbit IgG	1:100
CENP-A Antibody	2186	40 µl	Centromere	17 kDa	Rabbit IgG	1:400

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

**Description:** The Cellular Localization IF Antibody Sampler Kit provides an economical means for identification cellular organelles by fluorescence immunocytochemistry (IF-IC). This kit includes enough primary antibody to perform at least twenty IF-IC tests or four western mini-blot 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](http://www.cellsignal.com) for additional specificity/sensitivity information for individual kit components.

**Source/Purification:** Rabbit monoclonal and polyclonal antibodies are prepared by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to: the amino terminus of human β-tubulin, the sequence of human GAPDH, the sequence surrounding Ala51 of human calnexin, residues surrounding Lys29 of human COX IV, residues surrounding Gly190 of human Rab5 protein, the carboxy-terminal sequence of human histone H3, residues surrounding Pro671 of human NUP98, residues surrounding Thr298 of human fibrillarin, residues near the amino terminus of human Atg12, and human CENP-A protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

**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.

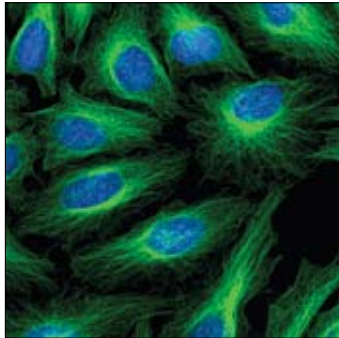
**Companion Products:**  
Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071  
Prestained Protein Marker, Broad Range (Premixed Format) #7720  
Biotinylated Protein Ladder Detection Pack #7727  
20X LumiGLO® Reagent and 20X Peroxide #7003  
Anti-rabbit IgG, HRP-linked Antibody #7074  
BSA #9998  
Nonfat Dry Milk #9999

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**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**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  
Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

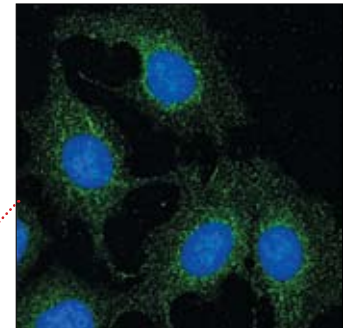


**Cytoskeletal staining**



*β-Tubulin (9F3) Rabbit mAb #2128 (green)*

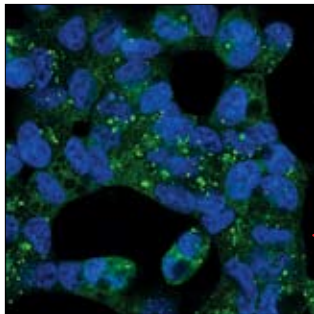
**Endosome staining**



*Rab5 Antibody #2143 (green)*

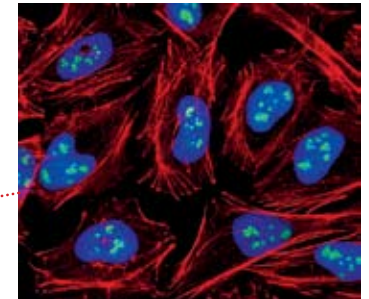
*Confocal immunofluorescent analysis of various cell types using the antibodies provided in the kit. Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5™ (fluorescent DNA dye).*

**Autophagosome staining**

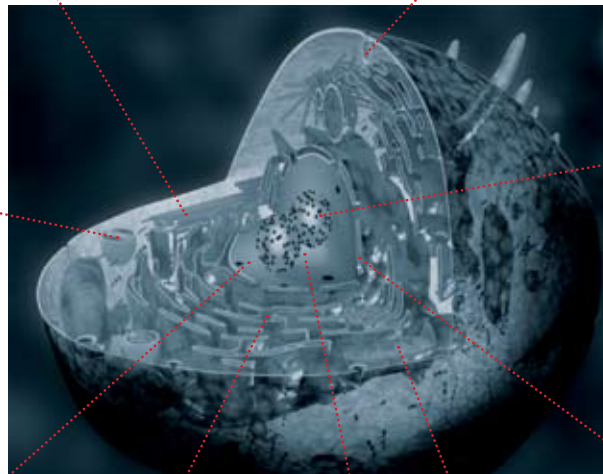


*Atg12 Antibody (Human Specific) #2010 (green)*

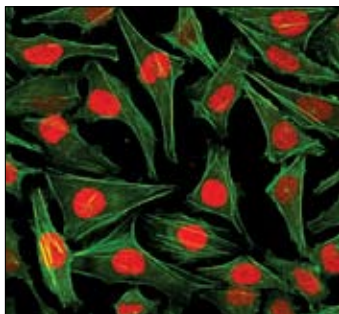
**Nucleolar staining**



*Fibrillarin (C13C3) Rabbit mAb #2639 (green)*

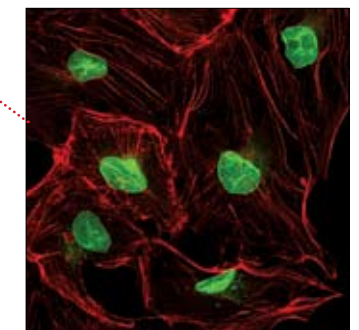


**Nuclear staining**



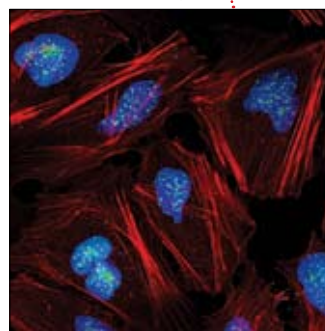
*Histone H3 Antibody #9715 (red)*

**Nuclear envelope staining**



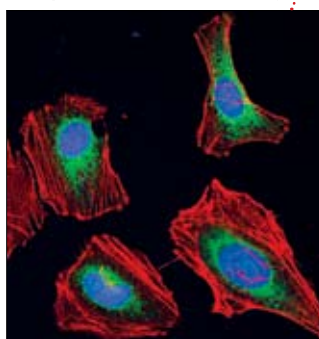
*NUP98 (C39A3) Rabbit mAb #2598 (green)*

**Centromere staining**



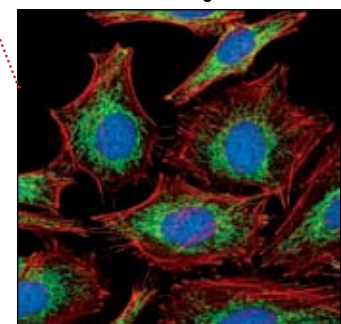
*CENP-A Antibody #2186 (green)*

**Endoplasmic reticulum staining**



*Calnexin Antibody #2433 (green)*

**Mitochondrial staining**



*COX IV (3E11) Rabbit mAb #4850 (green)*

## Immunofluorescence Protocol

**\*IMPORTANT:** Please refer to the **APPLICATIONS** section on the front page of the data sheet to determine **IF THIS PRODUCT** is validated and approved for the specific protocol you will be using.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1. 10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic ( $\text{Na}_2\text{HPO}_4$ ) and 2.4 g potassium phosphate, monobasic ( $\text{KH}_2\text{PO}_4$ ) to 1 L  $\text{dH}_2\text{O}$ . Adjust pH to 7.4.
- Formaldehyde, 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4°C in dark, dilute in PBS for use.
- Xylene
- Ethanol, anhydrous denatured, histological grade, 100% and 95%
- Distilled water ( $\text{dH}_2\text{O}$ )
- 1X PBS/0.3% Triton X-100 (PBS/Triton):** To prepare 1 L, add 100 ml 10X PBS to 900 ml  $\text{dH}_2\text{O}$ . Add 3 ml Triton X-100 and mix.
- 10 mM Sodium Citrate Buffer:** To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate ( $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) to 1 L  $\text{dH}_2\text{O}$ . Adjust pH to 6.0.
- 1X PBS, high salt (0.4M) (high salt PBS):** To prepare 1L, add 100 ml 10X PBS to 900 ml  $\text{dH}_2\text{O}$ . Add 23.38 g NaCl and mix.
- Fluorochrome-conjugated secondary antibody

**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.

- Prolong<sup>®</sup> Gold Antifade Reagent (Invitrogen, Eugene, OR, Cat# P36930)

### B Specimen Preparation

#### I. Cultured Cell Lines (IF-IC)

**IMPORTANT:** Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-IC)**.

**NOTE:** This general fixation protocol will work with most antibodies and cell lines. However, we recommend you try different IF/IC fixation methods (methanol or acetone alone, aldehyde alone, or combinations of these) to identify the optimal fixation protocol for each antibody and/or cell line.

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

- Rinse cells briefly in PBS.
- Aspirate PBS, cover cells to a depth of 2-3 mm with 2-4% formaldehyde in PBS.

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

- Allow cells to fix for 15 minutes at room temperature.
- Aspirate fixative, rinse three times in PBS for 5 minutes each.

**5. Methanol Permeabilization Step (if required, please refer to front page):** After formaldehyde fixation, cover cells with ice-cold 100% methanol (use enough to cover cells completely to a depth of 3-5 mm, **DO NOT LET CELLS DRY**), incubate cells in methanol for 10 minutes in freezer, rinse in PBS for 5 minutes.

- Proceed with Immunostaining section C.

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

**IMPORTANT:** Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-P)**.

Deparaffinization/Rehydration:

- Incubate sections in three washes of xylene for 5 minutes each.
- Incubate sections in two washes of 100% ethanol for 10 minutes each.
- Incubate sections in two washes of 95% ethanol for 10 minutes each.
- Rinse sections twice in  $\text{dH}_2\text{O}$  for 5 minutes each.

Antigen Unmasking:

- Place slides in room temperature 10 mM sodium citrate buffer pH 6.0.
- Bring slides to boiling in sodium citrate buffer using water bath or microwave, then maintain at 95-99°C for 10 minutes.
- Cool slides for 30 minutes on bench top.
- Rinse sections in  $\text{dH}_2\text{O}$  three times for 5 minutes each.
- Rinse sections in PBS for 5 minutes.
- Proceed with Immunostaining section C.

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

**IMPORTANT:** Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-F)**.

**NOTE:** Fresh frozen/unfixed sections should be fixed immediately in 2-4% formaldehyde as follows to preserve signaling epitopes.

- Cover sections with 2-4% formaldehyde in PBS

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

- Allow cells to fix for 15 minutes at room temperature.
- Rinse slides three times in PBS for 5 minutes each.

### 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.

- Block specimen in 5% normal serum from same species as secondary antibody (eg. normal goat serum, normal donkey serum) in PBS/Triton for 60 minutes.
- While blocking, prepare primary antibody by diluting as indicated on datasheet in PBS/Triton. You will need 50-100  $\mu\text{l}$  per section, 25-50  $\mu\text{l}$  per coverslip, chamber, or well (48 or 96 well plate).
- Aspirate blocking solution, apply diluted primary antibody.

**NOTE:** For double-labeling, prepare a cocktail of mouse and rabbit primary antibodies at their appropriate dilutions in PBS/Triton.

- Incubate overnight at 4°C.
- Rinse three times in PBS for 5 minutes each.

**OPTION:** To decrease background stain, rinse in high salt PBS for two minutes between second and third PBS rinses. Be aware, this may reduce specific staining of some antibodies.

**NOTE:** If using primary antibodies directly conjugated with AlexaFluor<sup>®</sup> fluorochromes, then skip to step C8.

- Incubate in fluorochrome-conjugated secondary antibody diluted in PBS/Triton for 1-2 hours at room temperature in dark.

**NOTE:** For double-labeling, prepare a cocktail of fluorochrome-conjugated anti-mouse and anti-rabbit primary antibodies at their appropriate dilutions in PBS/Triton.

- Rinse in PBS/high salt PBS as in step 5.
- Coverslip slides with Prolong<sup>®</sup> Gold Antifade Reagent or apply just enough to cover cells in multiwell plate.
- Seal slides by painting around edges of coverslips with nail polish.
- Examine specimens immediately using appropriate excitation wavelength, depending on fluorochrome for best results or store flat at 4°C in dark.