

Cellular Localization IF Antibody Sampler Kit

✓ 1 Kit
(9 x 40 µl)



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rev. 08/09/10

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 (C5C9) Rabbit mAb	2679	40 µl	Endoplasmic reticulum	90 kDa	Rabbit IgG	1:50
COX IV (3E11) Rabbit mAb	4850	40 µl	Mitochondria	17 kDa	Rabbit IgG	1:250
Rab5 (C8B1) Rabbit mAb	3547	40 µl	Endosomes	25 kDa	Rabbit IgG	1:100
Histone H3 (D1H2) XP™ Rabbit mAb	4499	40 µl	Nucleus	17 kDa	Rabbit IgG	1:200
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
LC3B (D11) XP™ Rabbit mAb	3868	40 µl	Autophagosomes	14, 16 kDa	Rabbit IgG	1:200
CENP-A Antibody	2186	40 µl	Centromere	17 kDa	Rabbit IgG	1:400

See 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 of cellular organelles by fluorescence immunocytochemistry (IF-IC). This kit includes enough primary antibody to perform at least twenty IF-IC tests or four 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 and polyclonal antibodies are prepared by immunizing animals with a synthetic peptide corresponding to: the amino terminus of human β-tubulin, the sequence of human GAPDH, the sequence 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 LC3B, and human CENP-A protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

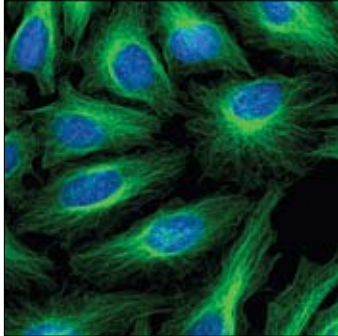
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C.
Do not aliquot the antibodies.

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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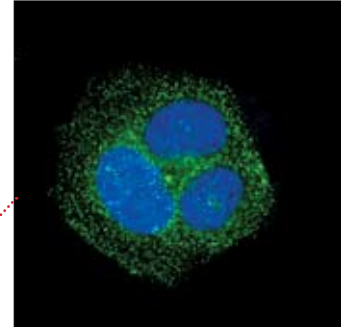


Cytoskeletal staining



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

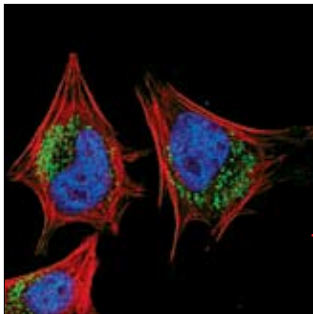
Endosome staining



Rab5 (C8B1) Rabbit mAb #3547 (green)

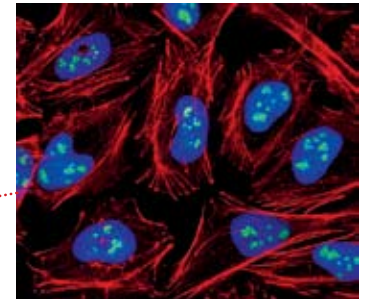
Confocal immunofluorescent analysis of various cell types using the antibodies provided in the kit. Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

Autophagosome staining

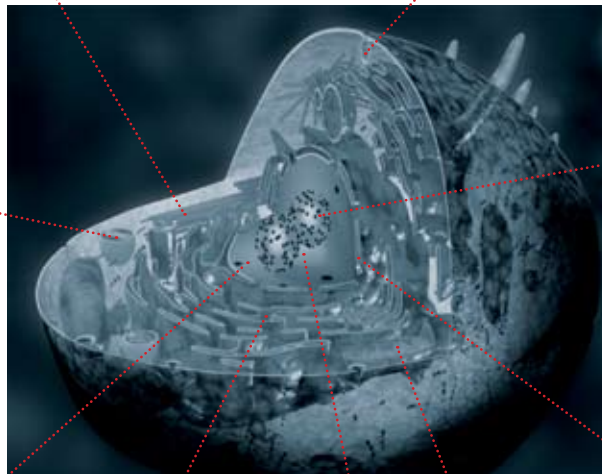


LC3B (D11) XP™ Rabbit mAb #3868 (green)

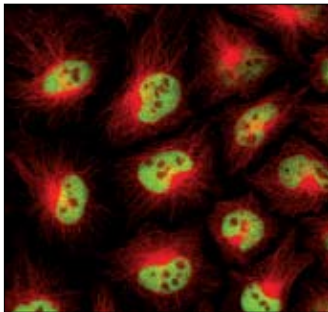
Nucleolar staining



Fibrillarin (C13C3) Rabbit mAb #2639 (green)

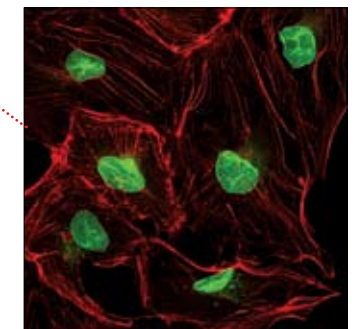


Nuclear staining



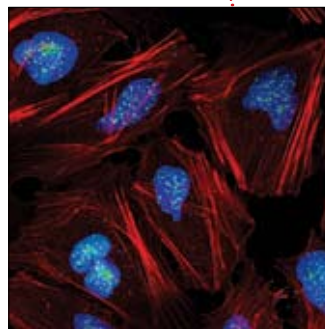
Histone H3 (D1H2) XP™ Rabbit mAb #4499 (green)

Nuclear envelope staining



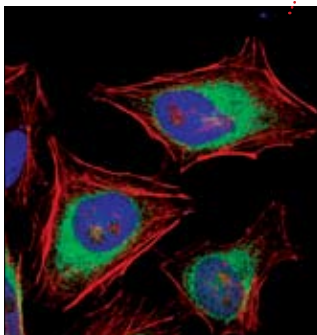
NUP98 (C39A3) Rabbit mAb #2598 (green)

Centromere staining



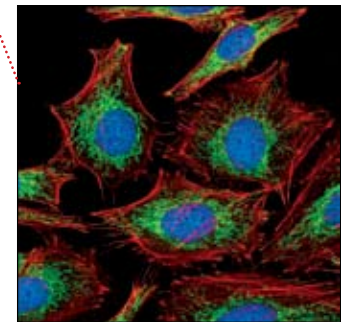
CENP-A Antibody #2186 (green)

Endoplasmic reticulum staining



Calnexin (C5C9) Rabbit mAb #2679 (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 (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O . 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 (dH_2O)
- 1X PBS/0.3% Triton X-100 (PBS/Triton):** To prepare 1 L, add 100 ml 10X PBS to 900 ml dH_2O . 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 dH_2O . 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 dH_2O . 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[®] 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 dH_2O 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 dH_2O 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 μl per section, 25-50 μl 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[®] 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[®] 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.