

Cat. #	Description	Product Type	Size	Applications	Species Reactivity	ICC Dilution
CM4371	Calnexin (N-terminal region)	Mouse mAb	50 µl	WB, E, ICC	Hu, Rt, Ms	1:50
CP2781	Caveolin-1 (N-terminal region)	Rabbit pAb	50 µl	WB, E, ICC	Hu, Rt, Ms, Ck	1:100
EM3471	Early Endosome Antigen 1 (EEA1)	Mouse mAb	50 µl	WB, E, ICC	Hu, Rt, Ms	1:50
GM3421	GM130 (C-terminal region)	Mouse mAb	50 µl	WB, E, ICC	Hu, Rt, Ms	1:50
HP4291	Histone H2B (C-terminus)	Rabbit pAb	50 µl	WB, E, ICC	Hu, Rt, Ms, F	1:200
HM4381	Hsp60 (N-terminal region)	Mouse mAb	50 µl	WB, E, ICC	Hu, Rt, Ms	1:100

Applications: WB = Western blot, E = ELISA, ICC = Immunocytochemistry, IP = Immunoprecipitation, IHC = Immunohistochemistry, FC = Flow Cytometry
 Species: H = Human, R = Rat, M = Mouse, C = Chicken, F = Fish, Fr = Frog, Rb = Rabbit

Kit Summary

The organelle marker kit can be used to co-localize proteins to specific organelles in cells. The kit includes a panel of antibodies that detect the following cell organelles:

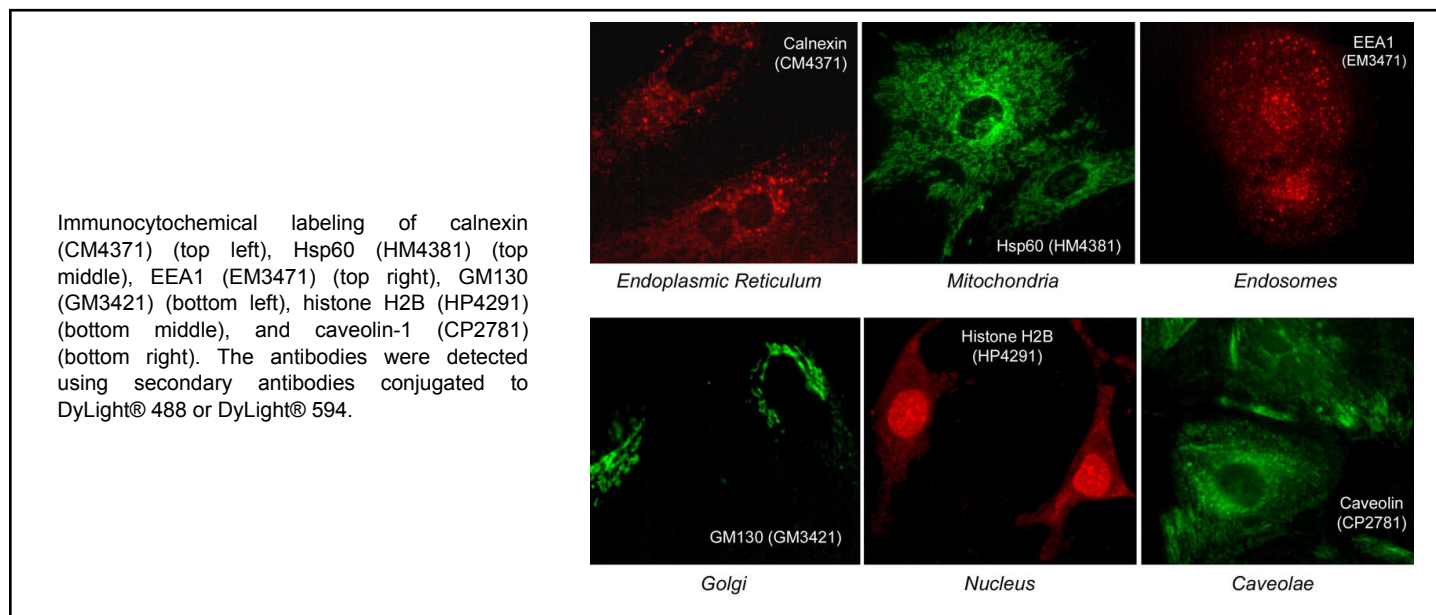
Caveolae = Caveolin 1
 Endoplasmic Reticulum = Calnexin
 Endosomes = EEA1
 Golgi = GM130
 Mitochondria = Hsp60
 Nucleus = Histone H2B

Background

A variety of subcellular compartments in eukaryotic cells perform specific activities related to cell function. The nucleus encloses DNA containing chromatin where gene transcriptions occurs. Endoplasmic reticulum, Golgi, and endosomes are involved in protein transport and protein post-translational modification and folding. Mitochondria contain proteins involved in cellular respiration, apoptosis, and free radical production. Caveolae are abundant cell-surface organelles involved in lipid regulation and endocytosis. Immunocytochemical co-labeling of newly discovered proteins with organelle marker antibodies is an important technique for determining protein function.

Buffers and Storage

Mouse monoclonal and rabbit polyclonal antibodies are supplied in phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. Store at -20°C . Stable for 1 year.



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Adherent Cell Fixation

1. Remove cell growth medium from culture plate containing cells, and rinse cells once with Hank's buffered saline solution (HBSS) or other rinse buffer acceptable for your cell type.
2. Fix cells with 4% Paraformaldehyde/0.2% NP-40 in HBSS for 30 minutes at room temperature.

Note: Some antibodies work better for immunocytochemistry using one of the following methods:

- A. Methanol/Acetone fixation: Fix and permeabilize in 1:1 Methanol/Acetone at -20°C for 10 min.
- B. Aldehyde/Acetone fixation: Fix cells with 4% Paraformaldehyde in HBSS for 30 minutes at room temperature, then permeabilize for 15 min. with 100% Acetone for at -20°C.

3. Remove fixation solution and rinse cells two times with phosphate buffered saline solution (PBS).
4. Block non-specific binding sites with 1% bovine serum albumin (BSA) in PBS for 30 minutes at room temperature.

Note: Normal animal serum (e.g. horse, goat) that matches the species of the secondary antibody can be substituted for BSA, if non-specific labeling occurs with certain secondary reagents.

Primary Antibody Labeling

5. Make primary antibody dilutions in 1% BSA in PBS, using the recommended dilution described in the table above. For some cell types, the optimal antibody dilution may need to be empirically determined. Titrations of 1:50 to 1:500 can be useful to determine the optimal dilution for each primary antibody.
6. Remove the blocking solution from step #4, then add primary antibody dilutions and incubate for 1-2 hours at room temperature.
7. After primary antibody probing, rinse cells three times with PBS.

Secondary Antibody Labeling

8. Make secondary dilutions in 1% BSA (or normal serum) in PBS.
9. Suggested dilutions for secondary antibodies used at ECM Biosciences:

RS3261	Goat anti-Rabbit Ig:DyLight® 488	(Green; Abs./Em. = 493/518)	1:200
MS3011	Goat anti-Mouse Ig:DyLight® 488	(Green; Abs./Em. = 493/518)	1:200
RS3271	Goat anti-Rabbit Ig:DyLight® 594	(Red; Abs./Em. = 593/618)	1:200
MS3031	Goat anti-Mouse Ig:DyLight® 594	(Red; Abs./Em. = 593/618)	1:200

10. Add secondary antibody to cells for 30 minutes at room temperature.
Note: Fluorescent secondary antibody labeling should be performed in the dark.
11. For long-term storage (months at 4°C), remove PBS and add SlowFade Gold (Invitrogen) to the cells and seal the slides or plates.

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