Digitonin treatment

- to disrupt the plasma membrane and wash out cytoplasmic, "unbound" ptoteins

Reference EMBO J., 20, 272-284 (2001)
"To remove cytosolic staining, cells were incubated in digitonin buffer (25 mM Hepes-KOH, pH 7, 125 mM KOAc, 2.5 mM $Mg(OAc)_2$, 0.004% digitonin) at room temperature for 2 minutes prior to fixation." 0.004%=40 μg/mL

Comment According to the result of HeLa cell expressing C-terminal fragment of mtGolgin-1, which localizes to TGN, I think 20 μg/mL digitonin was enough to see localization by immunofluorescence microscopy. Therefore you may reduce the concentration of digitonin to carry out this experiment.

Reagents

Digitonin stock solution
20 mg/mL digitonin in DMSO.
Stock at -20 C.

1 M Hepes-KOH, pH 7.0 (Hepes : FW 238.3)
Dissolve Hepes in 80% volume of water, adjust pH to 7.0 with KOH, and then volume up to 100% volume.

2 M KOAc (FW 98.14)

1 M $Mg(OAc)_2$ (FW 214.46)

KHM buffer

1 M Hepes-KOH, pH 7.0	x40 dilution
2 M KOAc	x16 dilution
1 M $Mg(OAc)_2$	x400 dilution

Digitonin in KHM buffer
Dilute digitonin stock solution to a final concentration of 20~40 μg/mL with KHM buffer.

Protocol

0) Prepare cells on coverslip.

1) Wash cells with KHM buffer.

2) Add 20~40 μg/mL digitonin in KHM buffer and incubate at room temperature for 2 minutes. Remove the solution.

3) Fix the cells : add 2% formaldehyde and leave at room temperature for 30 minutes. Wash the cells twice with PBS for 10 minutes.