

Making organelle panels (Organelle lab)

Day1

ManII-GFP::HeLa cells in 6cm w/~8CS

↓ 37°C, 5%CO₂, O/N

Day2

↓ ±BFA (1/200dil in culture medium for redistribution of Golgi enzymes to the ER)

or mito-tracker red (1/500 dil in HBSS), 30min, 37°C, 5%CO₂

Immunofluorescence

↓ cold MeOH 2min
 ↓ rehydration w/ PBS 15min
 ↓ Block w/ 4%BSA/PBS (filtered) 15min
 ↓ Primary Ab 15min
 ↓ wash x3 w/ PBS
 ↓ Secondary Ab +Hoechst 15min
 ↓ wash x3 w/ PBS
 ↓ rinse w/ DW

Mount (ProLong Gold+DAPI)

↓

FV1000, x60 oil, 2xzoom, 106.066µm/1024pixel

	BFA	Mitotracker	Primary	Secondary	Note
1.	+	-	anti-GM130	m568	Golgi, ER
2.	-	-	anti-GM130	m568	Golgi
3.	-	-	anti-HSP47	m568	Golgi, ER
4.	-	-	anti-EEA1	m568	Golgi, early endosomes
5.	-	-	anti-Lamp1	m568	Golgi, lysosomes
6.	-	+	-	-	Golgi, mitochondria

Cell lines

ManII-GFP::HeLa, HeLa cells stably expressing Golgi mannosidase-II (TM+stalk) fused by EGFP (Labmade)

Reagents

BFA 1mg/ml EtOH, Use 1:200, Sigma B7651

MitoTracker TR 1mg/ml DMSO, Use 1:500, Invitrogen #M7512

Primary antibodies

Anti-GM130, Use 1:50, BDBiosciences #610823

Anti-HSP47, Use 1:400, Stressgen

Anti-EEA1, Use 1:25, BDBiosciences #610457

Anti-Lamp1, Use 1:100, SCBT #sc-20011

Secondary antibodies

m568 (Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568): Use 1:100, Invitrogen #A11031

Mounting solution

ProLong Gold w/ DAPI (Invitrogen # P36935)

Transfection: http://www.okayama-u.ac.jp/user/organell/assets/Transfection_using_PEI.pdf