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Immunofluorescence imaging of cells expressing GolgiTAG (TMEM115-3HA) or HA-tagged non-Golgi annotated proteins

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ABSTRACT

Immunofluorescent (IF) microscopy is a powerful tool used in cellular and molecular biology to monitor the subcellular localisation of proteins. By combining the advantages of immunostaining and confocal light microscope, IF microscopy can be used to assess the colocalization of two or more proteins within the cell. Here, we describe a method that can be used to verify the correct localisation of HA-tagged Golgi proteins (like TMEM115-3HA, or GolgiTAG), by assessing their colocalisation with known Golgi markers such as GM130, GOLGIN97 and ACBD3. Furthermore, this method can be used to investigate whether HA-tagged, non-Golgi annotated proteins transiently expressed in cells localise at the Golgi, by assessing their colocalisation with ACBD3.

ATTACHMENTS

ius4bgrdf.docx

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MATERIALS TEXT

Cell lines:

- HEK293 (ATCC Catalog number CRL-1573, RRID:CVCL_0045)
- HeLa (ATCC Catalog number CCL-2, RRID:CVCL_0030)

Plasmids:

See Table 1. All plasmids are available from the MRC PPU Reagents and Services (https://mrcppureagents.dundee.ac.uk). (Note we purify plasmids using a QIAGEN HiSpeed® Plasmid Maxi kit [Lot# 166034460] following the manufacturer's instructions and ensure sterile reagents are used to avoid contamination).

Table 1: List of Plasmids. All plasmids are available from the MRC PPU Reagents and Services (https://mrcppureagents.dundee.ac.uk).

A	B	C
Plasmids	**Gene expressed**	**Plasmid Backbone**
DU72430	KIAA2013-HA	pcDNA5D
DU72445	TM9SF1-HA	pcDNA5D
DU72446	DIPK1A-HA	pcDNA5D
DU72447	C5orf22-HA	pcDNA5D
DU72466	SLC39A9-HA	pcDNA5D
DU72705	TMEM219-HA	pcDNA5D
DU72706	ABHD13-HA	pcDNA5D
DU72709	CCDC126-HA	pcDNA5D

Antibodies

- See Tables 2 and 3

ACBD3 (clone 2G2) **Sigma – Aldrich Catalog #WH0064746M1**

Golgin-97 (D8P2K) Rabbit mAb **Cell Signaling Technology Catalog #13192**

GM130 (D6B1) XP® Rabbit mAb **Cell Signaling Technology Catalog #12480**

HA **Sigma Catalog #11867423001**

Table 2: List of primary antibodies used for immunofluorescence staining.

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A	B	C	D	E
Antibody	**Company**	**Cat. Number (RRID)**	**Host species**	**Dilution for IF**
ACBD3 (clone 2G2)	Sigma	WH0064746M1 (RRID:AB_2220068)	Mouse	1:1000
GOLGIN97	Cell Signalling Technology	13192 (RRID:AB_2798144)	Rabbit	1:100
GM130	Cell Signalling Technology	12480 (RRID:AB_2797933)	Rabbit	1:3000
GCC185 (serum)	Pfeffer's lab	(PMID: 31663853)	Rabbit	1:500
HA	Sigma	11867423001 (RRID:AB_390918)	Rat	1:1000

Anti-Rabbit **Invitrogen - Thermo Fisher Catalog #A21206**

Anti-Rat **Invitrogen - Thermo Fisher Catalog #A21209**

anti-Mouse **Invitrogen - Thermo Fisher Catalog #A21202**

Table 3: List of fluorophore-conjugated secondary antibodies. All secondary antibodies are used at a 1:500 dilution.

A	B	C	D	E
Antibody	**Conjugated Fluorophore**	**Company**	**Cat. Number (RRID)**	**Host Species**
anti-Rat	Alexa 594	Invitrogen	A21209 (RRID:AB_2535795)	Donkey
anti-Rabbit	Alexa 488	Invitrogen	A21206 (RRID:AB_2535792)	Donkey
anti-Mouse	Alexa 488	Invitrogen	A21202 (RRID:AB_141607)	Donkey

Media and Reagents:

- **Growth Media:**

A	B
Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO 11960-085)	
Foetal Bovine Serum (FBS) (Sigma F7524 Batch# BCBW6817)	10% (v/v)
L-Glutamine (GIBCO 25030024)	1%
Penicillin-Streptomycin (GIBCO 15140122)	1%

DMEM, high glucose, no glutamine **Thermo Fisher Catalog #11960085**

Fetal Bovine Serum **Sigma Aldrich Catalog #F7524**

L-Glutamine (200mM) **Thermo Fisher Scientific Catalog #25030024**

Penicillin-Streptomycin **Gibco - Thermo Fisher Catalog #15140122**

- **Transfection media:**

Opti-MEM™ I Reduced Serum Medium **Gibco - Thermo Fischer Catalog #31985062**

- DPBS no calcium no magnesium **Gibco - Thermo Fischer Catalog #14190169**
-

PEI MAX® - Transfection Grade Linear Polyethylenimine Hydrochloride (MW 40000) **Polysciences Catalog #24765-1**

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- ☒Bovine Serum Albumin Fraction V **Sigma –**
- **Aldrich Catalog #10735094001**
- ☒Sodium azide **Sigma**
- **Aldrich Catalog #S2002**
- ☒Poly-L-lysine **Sigma –**
- **Aldrich Catalog #P4832**

Equipment

- Incubator with FPI-sensor system and display controller MB1 (BINDER GmbH. Model: CB150. Power Output: 1.40kW, 230V, 6.1 Amp)
- Leica TCS SP8 MP Multiphoton Microscope.
- See-saw rocker (VWR SSL4, or equivalent).

Consumables:

- ☒Nunc™ Cell-Culture Treated Multidishes, 6 well **Thermo**
- **Fisher Catalog #140675**
- ☒Cover glasses square **VWR international**
- **Ltd Catalog #631-0125**
- ☒Microscope slides SuperFrost® **VWR international**
- **Ltd Catalog #631-0114**
- ☒PIPETTE TIPS 100- 1000 µL BLUE SUITABLE FOR EPPENDORF STERILE 60 PIECES PER RACK **greiner bio-one Catalog #686271**
- ☒PIPETTE TIP 10 - 100 µL SUITABLE FOR EPPENDORF 96 PIECES / ST RACK **greiner bio-one Catalog #685261**

Seeding cells for immunofluorescence microscopy

1 Coat coverslips (sterilised in 100% ethanol prior to use) with poly-L-lysine by immersing the coverslips in poly-L-lysine solution for **01:00:00** . 1h

> **Note:** This is only necessary when using HEK293 cells.

2

Rinse the coated coverslips in media and place in a 6-well plate(one coverslip in each well).

3 Seed cells to 50-60% confluency in Growth media on coated coverslips from step 2.

4

Incubate **Overnight** .

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Note: For cells stably expressing GolgiTAG, skip to Step 4.

Transient transfection of cells for immunofluorescence microscopy

5

For each plasmid, prepare a transfection mix in a sterile **1.5 mL** Eppendorf tube containing:

- **2 µg** plasmid (see Table 1 for list of plasmids).
- **6 µL** [M]**1 mg/mL** PEI Max 40K (dissolved in distilled water and filter sterile).
- **300 µL** OptiMem.

6 30m

Incubate the transfection mix at **Room temperature** for **00:30:00** .

7

Add the transfection mix dropwise to the cells from step 3 using a P1000 sterile pipette.

8 1d

Incubate cells at **37 °C** for **24:00:00** .

Preparing cells for Immunofluorescence imaging 4h 10m

9 5m

Remove media completely using an aspirator and wash cells 3 times with **3 mL** PBS added wit 0.2% (w/v) BSA and 0.02% (w/v) sodium azide (**00:05:00** per wash on a see-saw rocker).

9.1 5m

Wash cells with **3 mL** PBS added wit 0.2% (w/v) BSA and 0.02% (w/v) sodium azide for **00:05:00** (1/3).

9.2 5m

Wash cells with **3 mL** PBS added wit 0.2% (w/v) BSA and 0.02% (w/v) sodium azide for **00:05:00** (2/3).

9.3 5m

Wash cells with **3 mL** PBS added wit 0.2% (w/v) BSA and 0.02% (w/v) sodium azide for **00:05:00** (3/3).

10 Fix cells by adding 4% (w/v) PFA in PBS.

11 10m

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Incubate at **Room temperature** for **00:10:00** .

12 Permeabilise cells with 1% (v/v) NP-40 in PBS + 0.2% (w/v) BSA + 0.02% (w/v) sodium azide.

13 Block with 1% (w/v) BSA in PBS at **Room temperature** for **00:30:00** . 30m

14 Prepare the primary antibody dilutions in 0.2% BSA (w/v) in PBS + 0.02% (w/v) sodium azide (See Table 2 for a list of antibodies and their working dilution).

Note: Primary antibodies raised in different species are combined for co-staining, as follows:
- Rat anti-HA and Rabbit anti-GM130
- Rat anti-HA and Rabbit anti-GOLGIN97
- Rat anti-HA and Mouse anti-ACBD3
- Rat anti-HA and Rabbit anti-GCC185

15 1h

Incubate cells at **Room temperature** with diluted primary antibodies for **01:00:00** .

Note: This should be done in a humid chamber to avoid samples drying out. Cover a glass plate with parafilm and add **20 µL** of primary antibody dilution to the relevant labelled area on the parafilm. Using tweezers, place each coverslip on the primary antibody solution (facing downward, so the cells are in contact with the antibody).

16 5m

Wash the coverslips 3 times with 0.2% (w/v) BSA in PBS + 0.02% sodium azide. (**00:05:00** per wash).

16.1 5m

Wash the coverslips with 0.2% (w/v) BSA in PBS + 0.02% sodium azide for **00:05:00** (1/3).

16.2 5m

Wash the coverslips with 0.2% (w/v) BSA in PBS + 0.02% sodium azide for **00:05:00** (2/3).

16.3 5m

Wash the coverslips with 0.2% (w/v) BSA in PBS + 0.02% sodium azide for **00:05:00** (3/3).

17 Prepare the secondary antibody dilutions in 0.2% BSA (w/v) in PBS + 0.02% (w/v) sodium azide (See Table 3 for a list of antibodies and their working dilution).

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18 1h

Incubate cells with diluted secondary antibodies (**20 µL** per coverslip) at **Room temperature** in the dark for **01:00:00** .

Note: As for the incubation with primary antibodies, this should be done in a humid chamber (see step 3.7).

19 5m

Wash cells 3 times in 0.2% (w/v) BSA in PBS + 0.02% (w/v) sodium azide. (**00:05:00** per wash).

19.1 5m

Wash cells in 0.2% (w/v) BSA in PBS + 0.02% (w/v) sodium azide for **00:05:00** (1/3).

19.2 5m

Wash cells in 0.2% (w/v) BSA in PBS + 0.02% (w/v) sodium azide for **00:05:00** (2/3).

19.3 5m

Wash cells in 0.2% (w/v) BSA in PBS + 0.02% (w/v) sodium azide for **00:05:00** (3/3).

20

Rinse cells by dipping briefly in MilliQ water and gently dry on Kleenex wipes.

21

Add a drop of Gold anti-glare oil (with DAPI for nucleus staining) (Invitrogen #2086915) on a microscope glass slide labelled with the sample ID.

22 30m

Mount cover slips from step 20 on the glass slide, ensuring that the side containing the cells is facing inward, making contact with the oil. Allow to dry for **00:30:00** in the dark.

23

Slides can be stored at **4 °C** or viewed immediately on a confocal microscope.

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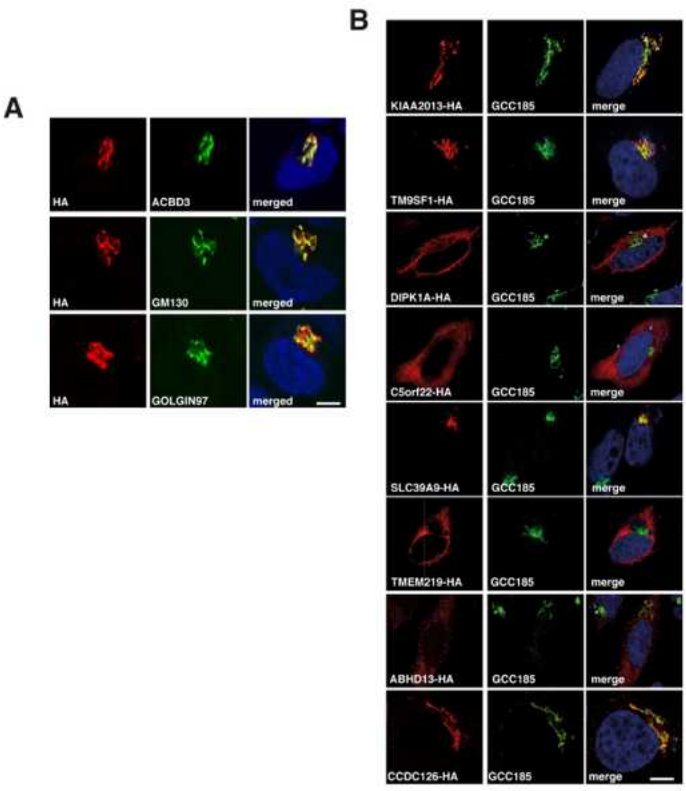

Figure 1: Immunofluorescence images of cells expressing various HA-tagged proteins. Cells were prepared for imaging using the methods described above. (A) HEK293 cells stably expressing GolgiTAG were co-immunostained for HA and Golgi markers such as ACBD3, GM130 and GOLGIN97. (B) HeLa cells transiently expressing HA-tagged proteins were co-immunostained for HA and a Golgi marker, GCC185. Scale bar is 2 µm.

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