Technical Data Sheet

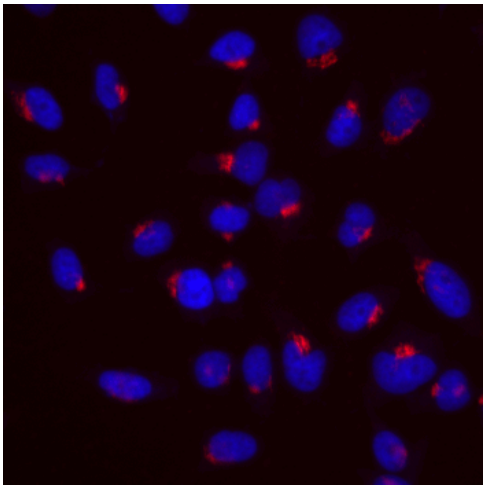
Alexa Fluor® 555 Mouse anti-GM130

Product Information

Material Number:	**560066**
Alternate Name:	Golgi autoantigen, Golgin 95, Golgin subfamily A member 2
Size:	100 tests
Vol. per Test:	5 µl
Clone:	35/GM130
Immunogen:	Rat GM130 aa. 869-982
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Confimed during development: Dog, Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

Maturation and post-translational modification of proteins occurs after their biosynthesis at the endoplasmic reticulum and their transport through the Golgi apparatus. The process involves the transport of vesicles carrying the proteins through a vectorial process of vesicle budding and fusion from the *cis*-compartment to the *medial*-compartment and the *trans*-compartment of the Golgi apparatus. The detergent insoluble fraction of the Golgi is named "matrix" and is required for proper morphology of the Golgi membranes. GM130 (Golgi matrix protein of 130 kDa) is a protein isolated from the Triton™ X-100-insoluble Golgi matrix and peripherally associated with the *cis*-compartment, as demonstrated by co-localization with syntaxin5. GM130 is homologous to the Golgi autoantigen golgin 95. GM130 interacts through its N-terminal domain with p115 and with the Golgi membranes at the C-terminal portion. Furthermore, the mitotic phosphorylation of GM130 blocks the interaction with p115. Thus, GM130 appears to function as a structural element of the Golgi apparatus that also provides attachment sites for membranes and other Golgi proteins. The 35/GM130 monoclonal antibody recognizes GM130, regardless of phosphorylation status.



Immunofluorescent staining of human cell lines. *HeLa cells (ATCC CCL-2) were cultured, fixed, permeabilized with Triton™ X-100, stained with Alexa Fluor® 555 Mouse anti-GM130 (pseudo colored red), and counter stained with Hoechst 33342 (pseudo colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 435 confocal bioimager with a 20x objective and merged using BD AttoVision™ software. This antibody also stains A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells, and it also worked with the Saponin and cold methanol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 555 under optimum conditions, and unreacted Alexa Fluor® 555 was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Bioimaging	Routinely Tested

Recommended Assay Procedure:

1. Seed the cells in appropriate culture medium at an appropriate cell density in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight to 48 hours.

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2. Remove the culture medium from the wells, and wash (one to two times) with 100 µl of 1× PBS.
3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 µl of 1× PBS.
5. Permeabilize the cells using either cold methanol (a), Triton™ X-100 (b), or Saponin (c):
    a. Add 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
    b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
    c. Add 100 µl of 1× Perm/Wash buffer (Cat. No. 554723) to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps.
6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 µl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.c.).
7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT.
8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10.
9. Add 50 µl of diluted antibody per well and incubate for 60 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies.
10. Remove the antibody, and wash the wells three times with 100 µl of wash buffer. An optional detergent wash (100 µl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps.
11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody.
12. After the final wash, counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
13. View and analyze the cells on an appropriate imaging instrument. Recommended filters for the BD Pathway™ instruments are:

Instrument	*Excitation*	*Emission*	*Dichroic*
BD Pathway 855	548/20	570LP	Fura/Fitc
BD Pathway 435	543/22	593/40	FF562

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
554723	Perm/Wash Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test when following the Recommended Assay Procedure. A Test is typically ~10,000 cells cultured in a well of a 96-well imaging plate.
2. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. Triton is a trademark of the Dow Chemical Company.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

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Gilbert J, Benjamin T. Uptake pathway of polyomavirus via ganglioside GD1a. *J Virol.* 2004; 78(22):12259-12267.(Clone-specific: Immunofluorescence)

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