Technical Data Sheet

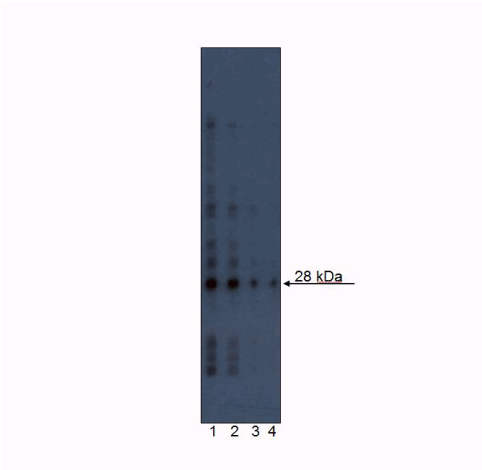
Polyclonal Rabbit Anti-Human D4-GDI

Product Information

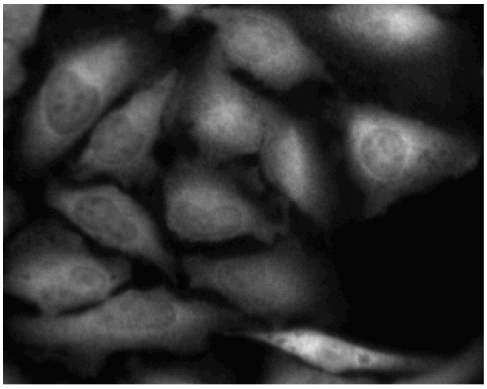
Material Number:	**556498**
Size:	0.1 ml
Immunogen:	Human D4-GDI aa. 4-21
Reactivity:	QC Testing: Human
Target MW:	28 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Guanine nucleotide dissociation inhibitors (GDIs) are negative regulators of Rho GTPases, a family of GTP-binding proteins which are thought to control the cytoskeletal and membrane changes that accompany cell death. D4-GDI and Rho-GDI are related GDI proteins, which differ in their cell-specific expression as well as in their ability to regulate GTPases. Rho-GDI is ubiquitously expressed and is a potent regulator of GTPases. By comparison, D4-GDI expression appears to be restricted to D4-GDI hematopoietic cells, and its regulatory activity appears to be lower than Rho-GDI. D4-GDI has been shown to be a substrate for the apoptotic associated proteases caspase-1 (ICE) and caspase-3 (CPP32). Active caspase-1 and caspase-3 cleave the 28 kD intact form of D4-GDI to 18 kD and 23 kD truncated forms, respectively. This process is believed to have significance with respect to the regulation of Rho GTPases during apoptosis. When used appropriately, the polyclonal rabbit anti-human D4-GDI antibody (Cat. No. 556498) recognizes the 28 kD intact form of human D4-GDI, but not the 18 and 23 kD cleaved fragments.



Western blot analysis of D4-GDI following apoptosis induction. *Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were treated for 4 hours with 4μM camptothecin (Sigma, Cat. No. C-9911). Cell lysatewas analyzed by western blotting using Polyclonal Rabbit anti-Human D4-GDI (Cat. No 556498) at 1:4,000 (Lane 1), 1:8,000 (Lane 2), 1:16,000 (Lane 3) and 1:32,000 (:Lane 4).*



Immunofluorescent staining of U-2 OS cells (Human osteosarcoma; ATCC HTB-96). *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10,000 cells per well. After overnight incubation, cells were stained using the Triton™ X-100 perm protocol and the Rabbit Anti-Human D4-GDI antibody. The second step reagent was FITC Goat Anti-Rabbit Ig (Cat. No. 554020). Images were taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and HeLa (ATCC CCL-2) cells which worked with both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at 4°C.

The polyclonal antibody was purified from antiserum by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

BD Biosciences

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Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/rabbit_poly_anti.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
554020	FITC Goat Anti-Rabbit Ig	0.5 mg	(none)
554021	HRP Goat Anti-Rabbit Ig	1.0 ml	(none)
550959	Jurkat Apoptotic Lysate Set I	500 µg	(none)
555670	Purified NA/LE Mouse Anti-Human CD95	0.5 mg	DX2
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
3. Triton is a trademark of the Dow Chemical Company.
4. 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.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Chong LD, Traynor-Kaplan A, Bokoch GM, Schwartz MA. The small GTP-binding protein Rho regulates a phosphatidylinositol 4-phosphate 5-kinase in mammalian cells. *Cell.* 1994; 79(3):507-513. (Biology)

Danley DE, Chuang TH, Bokoch GM.. Defective Rho GTPase regulation by IL-1 beta-converting enzyme-mediated cleavage of D4 GDP dissociation inhibitor. *J Immunol.* 1996; 157(2):500-503. (Biology)

Gorvel JP, Chang TC, Boretto J, Azuma T, Chavrier P. Differential properties of D4/LyGDI versus RhoGDI: phosphorylation and rho GTPase selectivity. *FEBS Lett.* 1998; 422(2):269-273. (Biology)

Na S, Chuang TH, Cunningham A, et al. D4-GDI, a substrate of CPP32, is proteolyzed during Fas-induced apoptosis. *J Biol Chem.* 1996; 271(19):11209-11213. (Biology)

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