Technical Data Sheet

Purified Rat anti-Mouse GRAIL

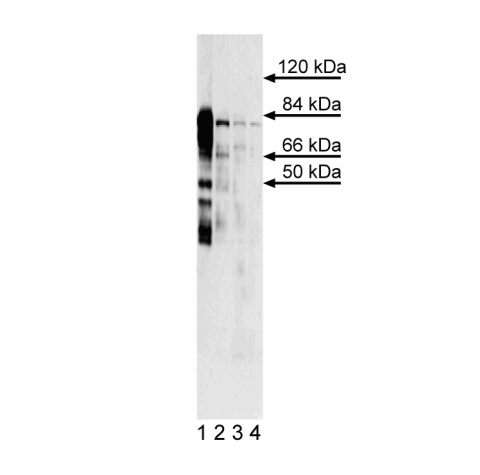
Product Information

Material Number:	**557799**
Alternate Name:	Goliath-related E3 ubiquitin-protein ligase 1, RNF128
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	H11-744
Immunogen:	Mouse GRAIL (full-length) Recombinant Protein
Isotype:	Rat (LOU) IgG2a, κ
Reactivity:	QC Testing: Mouse
Target MW:	62-66 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

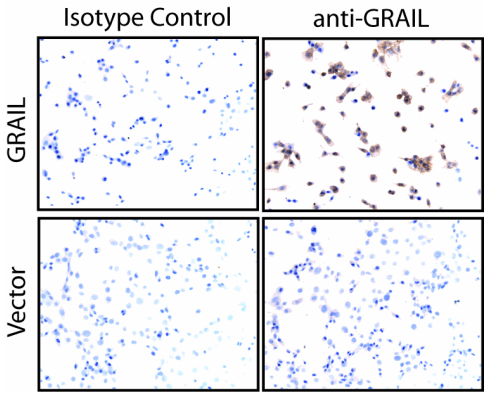
Description

The H11-744 antibody reacts with GRAIL (*G*ene *R*elated to *A*nergy *I*n *L*ymphocytes protein), a 428-amino acid glycoprotein with an apparent molecular weight of 62-66 kDa that is preferentially expressed in anergized CD4-positive CD25-negative T lymphocytes. The GRAIL protein sequence is homologous to several zinc RING finger-containing proteins, and thus, its gene has been designated *Rnf128* (RING finger protein 128). *Rnf128* transcripts are found in several mouse organs (including ovary, kidney, liver, brain, and heart), and transcription is induced rapidly (within 4 hours) in CD4-positive T cells upon in vitro induction of anergy. The expression of GRAIL correlates with the inhibition of IL-2 production in in vitro-anergized CD4-positive CD25-negative T cells, and GRAIL expression reduces IL-2 and IL-4 production in T-cell hybridomas activated by plate-bound anti-CD3 plus anti-CD28. GRAIL protein is localized in the recycling endosomal compartment, functions as an E3 ubiquitin ligase, and is required for anergy induction of CD4-positive T cells in vivo.

This product is sold under a license to patent number US 6,709,840.



Western blot analysis of mouse GRAIL. *Lysates from four cell lines were probed with 1 µg/mL of the purified rat anti-mouse GRAIL monoclonal antibody (clone H11-744). Lane 1: NIH/3T3 retrovirally transduced with a tagged Rnf128 gene. Lane 2: NIH/3T3 retrovirally transduced with the vector alone. Lane 3: Hepa 1-6 (mouse hepatoma, ATCC CRL-1830). Lane 4: NIH/3T3 (mouse embryo fibroblasts, ATCC CRL-1658). GRAIL is identified here on transfected cells as a doublet of 75-80 kDa as opposed to the 62-66 kDa size reported to be in cells expressing GRAIL endogenously.*



Immunocytochemical staining of mouse GRAIL. *Chamber slide cultures of NIH/3T3 retrovirally transduced with the Rnf128 gene (top row) and NIH/3T3 retrovirally transduced with vector alone (bottom row) were fixed with 2% paraformaldehyde. They were stained with either purified rat IgG2a, κ isotype control mAb (Cat. No. 559073, left column) or purified rat anti-mouse GRAIL (right column). A three-step staining procedure that employs biotin goat anti-rat IgG secondary antibody (Cat. No. 559286), streptavidin-HRP (Cat. No. 550946) and DAB (Cat. No. 550880) and hematoxylin counterstaining were used. GRAIL staining is localized in the cytoplasm of the GRAIL-expressing cells (top right panel). Original magnification: 20X.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Store undiluted at 4°C.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Application Notes

Application

Western blot	Routinely Tested
Immunocytochemistry (cytospins)	Tested During Development

Suggested Companion Products

Catalog Number	Name	Size	Clone
554017	HRP Goat Anti-Rat Ig	1.0 ml	Polyclonal
559073	Purified Rat IgG2a κ Isotype Control	0.25 mg	R35-95
559286	Biotin Goat Anti-Rat Ig	0.5 mg	Polyclonal
550946	Streptavidin HRP	50 ml	(none)
550880	DAB Substrate Kit	500 tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Anandasabapathy N, Ford GS, Bloom D, et al. GRAIL: An E3 ubiquitin ligase that inhibits cytokine gene transcription is expressed in anergic CD4+ T cells. *Immunity.* 2003; 18:535-547. (Biology: Western blot)

Ermann J, Szanya V, Ford GS, Paragas V, Fathman CG, Lejon K. CD4-positive CD25-positive T cells facilitate the induction of T cell anergy. *J Immunol.* 2001; 167:4271-4275. (Biology)

Joazeiro CAP, Weissman AM. RING finger proteins: mediators of ubiquitin ligase activity. *Cell.* 2000; 102:549-552. (Biology)

Mueller DL. E3 ubiquitin ligases as T cell anergy factors. *Nat Immunol.* 2004; 5(9):883-890. (Biology)

Seroogy CM, Soares L, Ranheim EA, et al. The gene related to anergy in lymphocytes, an E3 ubiquitin ligase, is necessary for anergy induction in CD4 T cells. *J Immunol.* 2004; 173:79-85. (Biology)

Soares L, Seroogy C, Skrenta H, et al. Two isoforms of otubain 1 regulate T cell anergy via GRAIL. *Nat Immunol.* 2004; 5:45-54. (Immunogen: Western blot)