Technical Data Sheet

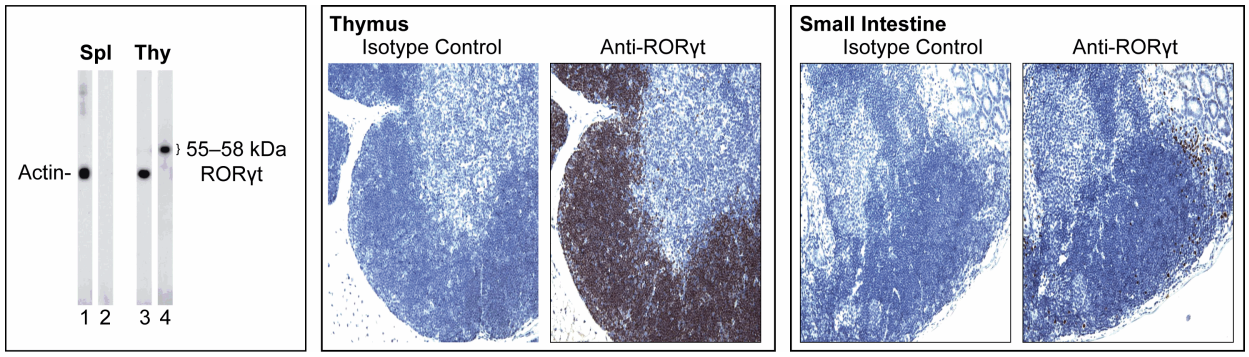
Purified Mouse anti-Mouse RORγt

Product Information

Material Number:	**562663**
Alternate Name:	RORγT; RORgt; RORgamma t; RORgammaT; Rorc2; Rorg; TOR; Thor; Nr1f3
Size:	50 µg
Concentration:	0.5 mg/ml
Clone:	Q31-378
Immunogen:	Mouse RORγt Recombinant Protein
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Testing: Mouse Not Reactive: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The Q31-378 monoclonal antibody recognizes RORgamma t (RORγt), an isoform of RORgamma (RORγ). RORγt is a DNA-binding transcription factor that belongs to the ROR/RZR orphan nuclear receptor family. RORγt is expressed exclusively by lymphoid cells including CD4+CD8+ thymocytes, peripheral CD4+ Th17 and CD8+ Tc17 cells, NKT cells and innate lymphoid cells such as lymphoid tissue inducer (LTi) cells. RORγt plays essential roles in thymopoiesis, T cell homeostasis, differentiation of effector T lymphocytes and the development of secondary lymphoid tissues including lymph nodes and Peyer's patches.



Analyses of RORγt Expression by Western bloting and Immunohistochemistry.

Left Panel: Western blot analyses of RORγt expression by mouse splenocytes and thymocytes. *Cell lysates from untreated mouse splenocytes (Spl; Lanes 1,2) and thymocytes (Thy; Lanes 3,4) (20 µg total cellular protein/lane) were electrophoresed (SDS-PAGE) and transferred to membranes. They were then probed with Purified Anti-Actin antibody (Cat. No. 612656/612657; Lanes 1,3 at 1 µg of antibody/ml) or Purified Mouse Anti-Mouse RORγt antibody (Clone Q31-378; Cat. No. 562663; Lanes 2,4 at 1 µg/ml). Mouse RORγt is identified as a protein band of ~56 kDa in the thymocyte lysate whereas actin is detected as ~42 kDa bands in the splenocyte and thymocyte lysates.*

Middle Panel: Immunohistochemical analysis of RORγt expressed in mouse thymocytes. *Following antigen retrieval with BD Retrievagen A Buffer (Cat. no. 550524), the formalin-fixed paraffin-embedded sections were stained with either Purified Mouse IgG2a, κ Isotype Control (Cat. No. 550339; Left Image) or Purified Mouse Anti-RORγt antibody (Q31-378; Right Image). This was followed by staining with Biotin Rat Anti-Mouse IgG2a secondary antibody (Cat. No. 550332), Streptavidin HRP (Cat. No. 550946) and a DAB Substrate Kit (Cat. No. 550880) with Hematoxylin counterstaining. The RORγt staining is nuclear as shown in the figures. Original magnification: 20x.*

Right Panel: Immunohistochemical analysis of RORγt expressed in mouse small intestine. *Sections of mouse small intestine were similarly stained for immunohistochemical analysis. 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	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Immunohistochemistry-frozen	Tested During Development
Immunohistochemistry-formalin (antigen retrieval required)	Tested During Development
Western blot	Tested During Development
Immunohistochemistry-paraffin	Not Recommended

Suggested Companion Products

Catalog Number	Name	Size	Clone
612656	Purified Mouse Anti-Actin Ab-5	50 µg	C4/actin
612657	Purified Mouse Anti-Actin Ab-5	150 µg	C4/actin
550524	Retrievagen A (pH 6.0)	1000 ml	(none)
550332	Biotin Rat Anti-Mouse IgG2a	1.0 ml	R19-15
550946	Streptavidin HRP	50 ml	(none)
550339	Purified Mouse IgG2a κ Isotype Control	1.0 ml	C1.18.4
550880	DAB Substrate Kit	500 tests	(none)
562574	Transcription Factor Buffer Set	100 tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
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. An isotype control should be used at the same concentration as the antibody of interest.

References

Bechtel S, Rosenfelder H, Duda A, et al. The full-ORF clone resource of the German cDNA Consortium. *BMC Biochem.* 2007; 8:399-410. (Biology)

Eberl G, Littman DR. The role of the nuclear hormone receptor RORgt in the development of lymph nodes and Peyer's patches. *Immunol Rev.* 2003; 195(195):81-90. (Biology)

Eberl G, Marmon S, Sunshine MJ, Rennert PD, Choi Y, Littman DR. An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. *Nat Immunol.* 2004; 5(1):64-73. (Biology)

Hamada H, Garcia-Hernandez MdlL, Reome JB, et al. Tc17, a unique subset of CD8 T cells that can protect against lethal influenza challenge. *J Immunol.* 2009; 182(6):3469-3481. (Biology)

He YW, Deftos ML, Ojala EW, Bevan MJ. RORgamma t, a novel isoform of an orphan receptor, negatively regulates Fas ligand expression and IL-2 production in T cells. *Immunity.* 1998; 9(6):797-806. (Biology)

Hirose T, Smith RJ, Jetten AM. ROR gamma: the third member of ROR/RZR orphan receptor subfamily that is highly expressed in skeletal muscle. *Biochim Biophys Acta.* 1994; 205(3):1976-1983. (Biology)

Ivanov, II, McKenzie BS, Zhou L, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell.* 2006; 126(6):1121-1133. (Biology)

Medvedev A, Yan ZH, Hirose T, Giguere V, Jetten AM. Cloning of a cDNA encoding the murine orphan receptor RZR/ROR gamma and characterization of its response element. *Gene.* 1996; 181(1-2):199-206. (Biology)

Unutmaz D. RORC2: the master of human Th17 cell programming. *Eur J Immunol.* 2009; 39(6):1452-1455. (Biology)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**