### **Technical Data Sheet**

# Alexa Fluor® 647 Mouse Anti-Mouse RORyt

### **Product Information**

Material Number: 562682

Alternate Name: RORyT; RORgt; RORgamma t; RORgammaT; Rorc2; Rorg; TOR; Thor; Nr1f3

 Size:
 50 µg

 Concentration:
 0.2 mg/ml

 Clone:
 Q31-378

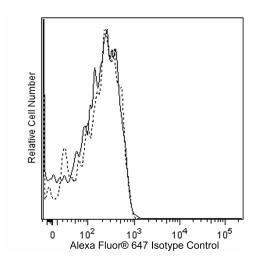
Immunogen: Mouse RORγt Recombinant Protein

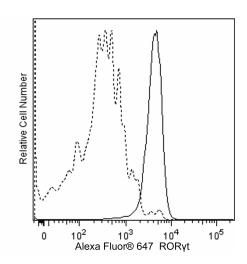
 $\begin{array}{ll} \textbf{Isotype:} & \textbf{Mouse IgG2a, } \kappa \\ \textbf{Reactivity:} & \textbf{QC Testing: Mouse} \end{array}$ 

Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

### Description

The Q31-378 monoclonal antibody recognizes mouse RORgamma t (ROR $\gamma$ t), an isoform of RORgamma (ROR $\gamma$ ). ROR $\gamma$ t is a DNA-binding transcription factor that belongs to the ROR/RZR orphan nuclear receptor family. ROR $\gamma$ t 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. ROR $\gamma$ t 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.





Multicolor flow cytometric analysis of RORyt expression in mouse thymocytes. BALB/c mouse thymocytes were stained with FITC Rat Anti-Mouse CD4 (Cat. No. 553047/553046/561835) and PE Rat Anti-Mouse CD8a (Cat. No. 555033/553032/561095) antibodies, and then fixed and permeabilized using the BD Pharmingen™ Transcription Factor Buffer Set (Cat. No. 562574/562725). The fixed and permeabilized cells were then stained with either Alexa Fluor® 647 Mouse IgG2a, κ Isotype Control (Cat. No. 557715) or Alexa Fluor® 647 Mouse Anti-Mouse RORyt antibody (Cat. No. 562682). The overlapping histograms show the levels of IgG2a Isotype Control (Left Panel) and RORyt (Right Panel) staining in CD4+ CD8-single-positive (dashed line histogram) or CD4+ CD8+ double-positive (solid line histogram) thymocytes. The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of intact cells. Flow cytometry was performed using a BD™ LSR II Flow Cytometry System.

### **Preparation and Storage**

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

### **Application Notes**

### Application

Intracellular staining (flow cytometry)

Routinely Tested

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#### **Recommended Assay Procedure:**

Optimal staining occurs when using the BD Pharmingen<sup>TM</sup> Alexa Fluor® 647 Mouse anti-Mouse RORyt antibody with cells that have been fixed and permeabilized with the BD Pharmingen<sup>TM</sup> Transcription Factor Buffer Set (Cat. No. 562574/562725).

### **Suggested Companion Products**

Catalog Number	Name	Size	Clone
557715	Alexa Fluor® 647 Mouse IgG2a, κ Isotype Control	100 tests	G155-178
562574	Transcription Factor Buffer Set	100 tests	(none)
562725	Transcription Factor Buffer Set	25 tests	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
562663	Purified Mouse anti-Mouse RORγt	50 μg	Q31-378
562197	Purified Mouse Anti-RORγt	0.1 mg	O28-835

### **Product Notices**

- Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 2 An isotype control should be used at the same concentration as the antibody of interest.
- Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols. 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.
- Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
- Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
- 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.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

#### 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)

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