Phenotyping on the front lines

Flow cytometry antibodies for identifying tissue-resident memory T cells.

Identified about a decade ago, tissue-resident memory T cells (T_{RM}) provide antigen-specific protection from pathogens and viruses in peripheral tissues. Characterized by their residency in tissues and distinct inability to recirculate, T_{RM} have been identified in lung, skin, liver, brain, intestinal, and mucosal tissues [1-4]. While initial studies focused on their persistence at sites of previous infection and their long-lived role in combating reinfection (through immediate effector function and accelerated recruitment of circulating immune cells), T_{RM} have also been reported to accumulate in the tumor microenvironment, including those of epithelial (ovarian, pancreatic, colorectal, and lung) and nonepithelial (malignant glioma and melanoma) origin [5].

The efficacy of immune checkpoint inhibitors (anti–PD-1, anti–PD-L1, and others) in cancer treatment has led researchers to postulate that T_{RM} are key players in the tumor microenvironment, capable of initiating and maintaining antitumor responses due to their high levels of expression of inhibitory receptors. Potential mechanisms of action include rapid and local antigen-specific proliferative responses, with expression of effector molecules to promote inflammation and immune cell recruitment and differentiation (IFNγ, TNFα, IL-2, IL-17) and to direct target-cell lysis (perforin, granzyme B) [6,7]. Therefore, increased activation of T_{RM} is thought to be a fruitful strategy for enhancing current immunotherapy approaches and vaccination efficacy. In this article, we will review common T_{RM} markers and address the development of T_{RM} flow cytometry panels. Thermo Fisher Scientific offers a wide variety of Invitrogen™ primary antibodies and antibody conjugates for flow cytometry that recognize positive and negative markers for T_{RM} (Table 1).

Key cell-surface markers for T_{RM}

The peripheral blood T cell population contains a variety of subsets that can be identified by expression of CD45RA, CCR7 (CD197), and CD62L (L-selectin). These subsets include naïve ($CD45RA^+ CCR7^+ CD62L^+$), central memory (T_{CM}; $CD45RA^- CCR7^+ CD62L^+$), and effector memory (T_{EM}; $CD45RA^- CCR7^- CD62L^-$) T cells, as well as terminally differentiated effector memory cells re-expressing CD45RA (T_{EMRA}; $CD45RA^+ CCR7^- CD62L^-$) [8]. T_{EM} play an important role in immune surveillance and are a major subset of both $CD4^+$ and $CD8^+$ T cell populations found in peripheral tissues [7,9]. In contrast, T_{CM} survey secondary lymphoid organs for cognate antigen, while naïve cells are typically localized to the blood, spleen, and lymph nodes [8].

CD69: Human T_{RM} are typically associated with the expression of the surface markers CD69, CD49a (integrin α1), and CD103 (integrin αE); these markers, along with CD44, are useful for studying mouse T_{RM}. Although CD69 is a marker of early T cell activation, most T_{RM} express CD69 under steady-state conditions, without expression of other activation markers such as CD25, CD38, and HLA-DR [10]. CD69 is therefore an important distinguishing cell-surface marker constitutively expressed on T_{RM} in most tissues, and it functions as a critical antagonist of S1PR1 (CD363) activity [11]. The $CD69^+ T_{RM}$ population is phenotypically and transcriptionally distinct from recirculating $CD69^-$ memory T cells in both tissues and blood, each having a defined gene expression signature that includes molecules associated with adhesion, migration, and regulation [6,7].

CD49a: CD49a (integrin α1) is also an important marker expressed in some T_{RM}, as it acts with CD29 (integrin β1) to form the heterodimeric molecule VLA-1, which can bind collagen and laminin and promotes tissue residency [5,12].

CD103: CD103 (integrin αE) expression in T_{RM} is variable [7]. CD103 is upregulated after exposure to TGF-β, and it complexes with integrin β7 on the T cell surface to allow adherence through binding to CD324 (E-cadherin) on epithelial cells [11]. CD103 expression is restricted to $CD8^+ T_{RM}$ in mucosal sites and skin [7,8,9]. T_{RM} that exist outside of epithelial tissues generally lack CD103 expression, although they may express other adhesion molecules such as LFA-1, which is a heterodimeric integrin composed of CD11a (LFA-1α) and CD18 (LFA-1β). In mice, LFA-1 is present on $CD103^-$ liver-resident T_{RM} and is thought to allow binding of CD54 (ICAM-1) on liver sinusoidal endothelial cells. It is not yet known if this marker is expressed on human liver-resident T_{RM} [13].

CD44: CD44 is a C-lectin–containing glycoprotein that is expressed on leukocytes and other cell types and serves as a receptor for hyaluronic acid (HUA), which is an extracellular matrix component produced by vascular endothelial cells and other immune cells [11]. CD44 also binds to other matrix proteins like fibronectin, laminin, and collagen [11]. In mice, CD44 is considered a core marker with a functional role
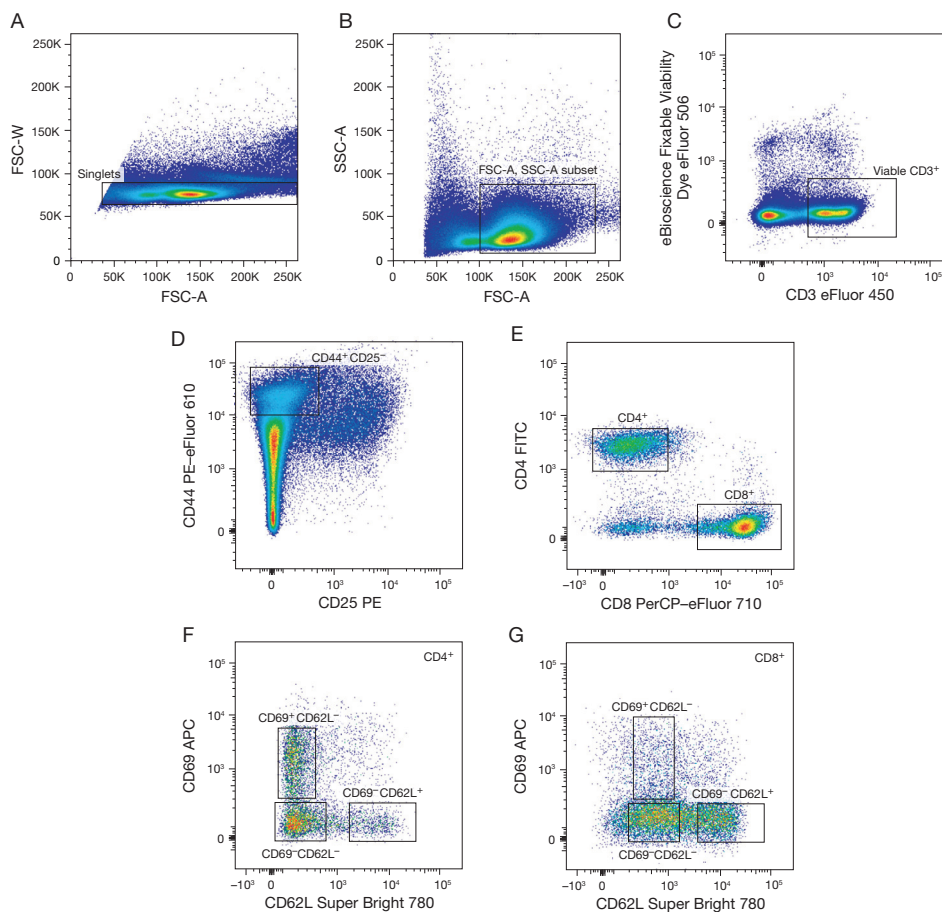
Figure 1. Example of a flow cytometry panel for T$_{RM}$. C57BL/6 mouse lymph node cells were stained with Invitrogen™ eBioscience™ CD3 eFluor™ 450, CD44 PE–eFluor™ 610, CD25 PE, CD62L Super Bright 780, CD4 FITC, CD8 PerCP–eFluor™ 710, and CD69 APC monoclonal antibodies. Viability was determined using Invitrogen™ eBioscience™ Fixable Viability Dye eFluor™ 506 (Cat. No. 65-0866-18). For analysis, viable CD3$^+$ T cells **(C)** were gated from singlet-gated **(A)** mouse lymph node cells **(B)**. These CD3$^+$ T cells were further gated by CD44$^+$ and CD25$^-$ expression (memory and activation/Treg phenotype markers, respectively) **(D)**, and then into CD4$^+$ and CD8$^+$ subsets **(E)**. CD69 and CD62L expression identifies T$_{RM}$ cells (CD69$^+$CD62L$^-$), T$_{CM}$ cells (CD69$^-$CD62L$^+$), and T$_{EM}$ cells (CD69$^-$CD62L$^-$) in the CD4$^+$ population **(F)** and the CD8$^+$ population **(G)**. **For Research Use Only. Not for use in diagnostic procedures. Not for resale.** Super Bright Polymer Dyes are sold under license from Becton, Dickinson and Company.

in T$_{RM}$ biology. Its expression, however, does not distinguish the T$_{RM}$ subset from other CD8$^+$ T cell populations, and it is mainly used as a marker of previous T cell activation, as it also labels T$_{CM}$ and T$_{EM}$ [11]. The role of CD44 in T$_{RM}$ may include regulation of cell–cell interactions, cell adhesion, migration, or lymphocyte activation.

T$_{RM}$ surface marker signature

The Farber lab at the Columbia University Medical Center has reported a core T$_{RM}$ surface marker signature consistent across tissues and diverse human donors and expressed in both CD4$^+$ and CD8$^+$ T cells [7]. In their approach, T$_{RM}$ are identified as CD69$^+$, while T$_{EM}$ are CD69$^-$. When compared to CD69$^-$ T$_{EM}$, CD69$^+$ T$_{RM}$ show upregulated expression of CD103 (integrin αE), CD49a (integrin α1), CRTAM (CD355),

CD186 (CXCR6), CD279 (PD-1), MKP3 (DUSP6), and IL-10. Notably, expression of CD186 (CXCR6) allows for migration into peripheral tissues. Furthermore, CD69$^+$ T$_{RM}$ show downregulated expression of S1PR1, CD62L, CX3CR1, and KLF2 relative to CD69$^-$ T$_{EM}$, a phenotype consistent with the retention of T$_{RM}$ within tissues [7,9].

Other markers of interest expressed specifically in CD8$^+$ CD69$^+$ T$_{RM}$ are ICOS and IRF4 [7]. CD101 is also upregulated on CD8$^+$ T$_{RM}$, as compared with CD4$^+$ T$_{RM}$ in the lung and spleen, and may be involved in inhibition of T cell activation and proliferation [7,9]. CD28 and CD127 (IL-7R) may be useful for delineating cell activation and homeostasis [4]. T$_{RM}$ development and maintenance are regulated by CD122 (IL-15R) and CD127 (IL-7R), and the latter receptor is expressed on a majority of CD69$^+$ T$_{RM}$ [6]. Other homing and retention markers may ⟶

be utilized to distinguish T_{RM} in specific compartments, such as CLA (CD162, PSGL-1), CCR8, CCR10, FABP4, and FABP5 on skin-resident T_{RM}, and CCR9 and integrin α4β7 (LPAM-1) on intestine-resident T_{RM} [5].

The study of transcriptional regulation in T_{RM} is ongoing. Perhaps as a function of their diversified tissue distribution with myriad metabolic niches, T_{RM} are currently thought to rely on combinations of transcription factors, and a unifying lineage-restricted master regulator has not yet been identified [11]. That said, several transcription factors have been identified as important for T_{RM} tissue residency. T_{RM} are reported to downregulate the T-box transcription factors EOMES (negative) and T-bet (low) following TGF-β signaling [5,6,14]. RUNX3 enhances granzyme B and CD103 expression, whereas NOTCH1 appears to regulate metabolism in T_{RM} [6]. Other transcription factors may contribute to tissue residency programs, such as BLIMP-1 and HOBIT, which can repress CCR7, S1PR1, KLF2, and TCF-1 expression (although some reports suggest the role of HOBIT in T_{RM} may be limited) [5,9]. Current research is focused on identifying the components of the general and tissue-specific signaling pathways that regulate T_{RM}.

Flow cytometry panels for T_{RM}

Taking these data into consideration, flow cytometry panels for T_{RM} may include a core set of markers such as CD45, CD69, CD3, CD4, CD8, CD279 (PD-1), CD103 (integrin αE), CD186 (CXCR6), CD19 (negative), CD45RA (negative), CCR7 (negative), and CD62L (negative) (Figure 1). Further clarity would be provided by inclusion of CX3CR1 and S1PR1 markers in the panels [6]. CD44 is useful in mouse T_{RM} panels [9]. T_{RM} may also upregulate CD28 and CD127 (IL-7R), although this upregulation appears to depend on tissue localization [7,8]. Further

memory phenotypes may be identified using KLRG1 and CD27. Tissue-specific homing markers, such as LFA-1, CCR8, CCR10, CLA, FABP4, FABP5, CCR9, and integrin α4β7 (LPAM-1) may also be of interest [6].

Antibody selection tools for your flow cytometry panels

T_{RM} are an important and still-emerging subset in oncology, potentially other disease research, and vaccine design. Further studies are needed to illuminate their development and roles in disease protection and progression. Table 1 lists selected Invitrogen™ flow cytometry antibodies and antibody conjugates for the study of T_{RM}. Search our complete portfolio of primary and secondary antibodies for flow cytometry, immunofluorescence, western blotting, ELISAs, and other applications at **thermofisher.com/antibodies**. ∎

References

1. Mami-Chouaib F, Tartour E (2019) *Front Immunol* 10:1018. PMID 31191515
2. Mueller SN, Mackay LK (2016) *Nat Rev Immunol* 16:79–89. PMID 26688350
3. Schenkel JM, Fraser KA, Masopust D (2014) *J Immunol* 192:2961–2964. PMID 24600038
4. Thome JJ, Yudanin N, Ohmura Y et al. (2014) *Cell* 159:814–828. PMID 25417158
5. Corgnac S, Boutet M, Kfoury M et al. (2018) *Front Immunol* 9:1904. PMID 30158938
6. Behr FM, Chuwonpad A, Stark R et al. (2018) *Front Immunol* 9:1770. PMID 30131803
7. Kumar BV, Ma W, Miron M et al. (2017) *Cell Rep* 20:2921–2934. PMID 28930685
8. Thome JJ, Farber DL (2015) *Trends Immunol* 36:428–435. PMID 26072286
9. Steinbach K, Vincenti I, Merkler D (2018) *Front Immunol* 9:2827. PMID 30555489
10. Sathaliyawala T, Kubota M, Yudanin N et al. (2013) *Immunity* 38:187–197. PMID 23260195
11. Topham DJ, Reilly EC (2018) *Front Immunol* 9:515. PMID 29632527
12. Ray SJ, Franki SN, Pierce RH et al. (2004) *Immunity* 20:167–179. PMID 14975239
13. McNamara HA, Cai Y, Wagle MV et al. (2017) *Sci Immunol* 2:eaaj1996. PMID 28707003
14. Mackay LK, Wynne-Jones E, Freestone D et al. (2015) *Immunity* 43:1101–1111. PMID 26682984

The Flow Cytometry Panel Builder—A tool for all flow cytometrists

Whether you are a novice or an expert, designing a panel for flow cytometry is a highly complex process. If you are a beginner, let the Invitrogen™ Flow Cytometry Panel Builder simplify your panel building by making the pairing of markers and fluorophores quick and easy using a highly visual format. Are you an expert? Then you will appreciate using the Flow Cytometry Panel Builder to efficiently review the spectral signals and filters per laser line and check fluorophore spillover values per channel. With access to over 13,000 antibodies for flow cytometry, this tool allows quick identification of antibodies for flow cytometry panels. Get started building your panel today at **thermofisher.com/flowpanel**.
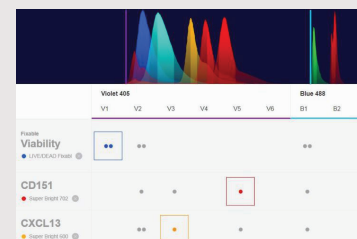
Table 1. Invitrogen™ flow cytometry antibodies and selected antibody conjugates for the study of tissue-resident memory T cells.

Species	Marker type	Marker	Location	Notes	Clone (selected* Cat. No.)
Mouse	Core marker	CD69	Surface	Found on most T_{RM}; also expressed by activated cells	H1.2F3 (12-0691-82)
	General phenotype (not tissue-specific)	CD45	Surface	General lymphocyte marker	30-F11 (12-0451-82)
		CD3	Surface	General T cell marker	145-2C11 (12-0031-82); 17A2 (12-0032-82); eBio500A2 (12-0033-82)
		CD4	Surface	CD4+ T cell subset marker	GK1.5 (12-0041-82); RM4-5 (12-0042-82); RM4-4 (12-0043-82)
		CD8	Surface	CD8+ T cell subset marker	53-6.7 (12-0081-82)
		CD279 (PD-1)	Surface	Highly expressed on T_{RM}	J43 (12-9985-82); RMP1-30 (12-9981-82)
		CD101	Surface	Subsets of T_{RM}	Moushi101 (12-1011-82)
		CD11a	Surface	Subunit of LFA-1	M17/4 (12-0111-82)
		CD44	Surface	Expression gradient: naïve < effector < memory T cells	IM7 (12-0441-82)
		CD127 (IL-7R)	Surface	Subsets of T_{RM}	A7R34 (12-1271-82)
		KLRG1	Surface	Negative or low expression on T_{RM}	2F1 (12-5893-82)
		CCR7 (CD197)	Surface	Negative on T_{RM}; expressed by T_{CM}	4B12 (12-1971-82)
		CD62L (L-selectin)	Surface	Negative on T_{RM}; expressed by T_{CM}	MEL-14 (12-0621-82)
		CD45.1 CD45.2	Surface	May be used for parabiosis studies	A20 (12-0453-82) 104 (12-0454-82)
		Blimp-1	Nuclear		5E7 (12-9850-82)
	Mucosal and barrier sites	CD103 (integrin αE)	Surface	Subsets of T_{RM}	2E7 (12-1031-82)
	Skin and liver	CD186 (CXCR6)	Surface	Subsets of T_{RM}	DANID2 (12-9186-82)
	Skin	CD183 (CXCR3)	Surface		CXCR3-173 (12-1831-82)
	Liver	LFA-1	Surface		M17/4 (12-0111-82); M18/2 (12-0181-82)
Human	Core marker	CD69	Surface	Found on most T_{RM}; also expressed by activated cells	FN50 (12-0699-42)
	General phenotype (not tissue-specific)	CD45	Surface	General lymphocyte marker	HI30 (12-0459-42); 2D1 (12-9459-42)
		CD3	Surface	General T cell marker	UCHT1 (12-0038-42); SK7 (12-0036-42); OKT3 (12-0037-42); HIT3a (12-0039-42)
		CD4	Surface	CD4+ T cell subset marker	RPA-T4 (12-0049-42); OKT4 (12-0048-42); SK3 (12-0047-42)
		CD8	Surface	CD8+ T cell subset marker	RPA-T8 (12-0088-80); OKT8 (12-0086-42); HIT8a (12-0089-42); SK1 (12-0087-42)
		CD45RA	Surface	Naïve T cell marker; negative on T_{RM}	HI100 (12-0458-42)
		CD45RO	Surface	Memory T cell marker	UCHL1 (12-0457-42)
		CD279 (PD-1)	Surface	Highly expressed on T_{RM}	MIH4 (12-9969-42); eBioJ105 (14-2799-80)
		CD127 (IL-7R)	Surface	Subsets of T_{RM}	eBioRDR5 (12-1278-42)
		NOTCH1	Intracellular	Possible T_{RM} metabolic marker	mN1A (12-5785-82)
		CRTAM (CD355)	Surface	Upregulated on some T_{RM} subsets (adhesion molecule)	Cr24.1 (12-3559-42)
		S1PR1 (CD363)	Surface	Downregulated on T_{RM}	SW4GYPP (50-3639-42)
		CX3CR1	Surface	Downregulated on T_{RM} vs. circulating T_{RM}	2A9-1 (12-6099-42)
		CCR7 (CD197)	Surface	Negative on T_{RM}; expressed by T_{CM}	3D12 (12-1979-42)
		CD62L (L-selectin)	Surface	Negative on T_{RM}; expressed by T_{CM}	Dreg-56 (12-0629-42)
		CD101	Surface	Subsets of T_{RM}	BB27 (14-1019-82)
	Mucosal and barrier sites	CD103 (integrin αE)	Surface	Subsets of T_{RM}	B-Ly7 (12-1038-42)
	Lung and skin	CD49a (integrin α1)	Surface		TS2/7 (46-9490-42)
	Lung	CD183 (CXCR3)	Surface		CEW33D (12-1839-42)
	Skin	CD194 (CCR4) CLA	Surface		D8SEE (12-1949-42) HECA-452 (50-9857-82)
	Lymph node	CD184 (CXCR4) CD185 (CXCR5)	Surface		12G5 (12-9999-42) MU5UBEE (12-9185-42)
	Liver	LFA-1	Surface		HI111 (11-0119-42); 6.7 (12-0189-42); R3.3 (BMS103FI)

*Antibodies are available as several different conjugates and in multiple packagings. Go to thermofisher.com/antibodies to see a complete listing.