Revision 1

Store at -20C

#43049

Human Exhausted CD8+ T Cell IHC Antibody Sampler Kit

1 Kit (9 x 20 microliters)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
CD3ε (D7A6E™) XP® Rabbit mAb	85061	20 µl	23 kDa	Rabbit IgG
CD8α (D8A8Y) Rabbit mAb	85336	20 µl	29 kDa	Rabbit IgG
Tox/Tox2 (E6I3Q) Rabbit mAb	73758	20 µl	60-80 kDa	Rabbit IgG
TCF1/TCF7 (C63D9) Rabbit mAb	2203	20 µl	48, 50 kDa	Rabbit IgG
Granzyme B (D6E9W) Rabbit mAb	46890	20 µl	30 kDa	Rabbit IgG
PD-1 (Intracellular Domain) (D4W2J) XP® Rabbit mAb	86163	20 µl	52-65 kDa	Rabbit IgG
TIGIT (E5Y1W) XP® Rabbit mAb	99567	20 µl	18, 30-40 kDa	Rabbit IgG
TIM-3 (D5D5R™) XP® Rabbit mAb	45208	20 µl	45-70 kDa	Rabbit IgG
LAG3 (D2G4O) XP® Rabbit mAb	15372	20 µl	60-80 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

Description

The Human Exhausted CD8+ T Cell IHC Antibody Sampler Kit provides an economical means of characterizing the extent of exhaustion in T cells in formalin-fixed, paraffin-embedded tissue samples.

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Background

Cluster of Differentiation 3 (CD3) is a multiunit protein complex expressed on the surface of T cells that directly associates with the T cell receptor (TCR). CD3 is composed of four polypeptides: ζ, γ, ε, and δ. Engagement of the TCR complex with antigens presented in major histocompatibility complexes induces tyrosine phosphorylation in the immunoreceptor tyrosine-based activation motif (ITAM) of CD3 proteins. CD3 phosphorylation is required for downstream signaling through ZAP-70 and p85 subunit of PI-3 kinase, leading to T cell activation, proliferation, and effector functions (1). CD8 is a transmembrane glycoprotein expressed primarily on cytotoxic T cells, but has also been described on a subset of dendritic cells in mice (2,3). On T cells, CD8 is a co-receptor for the TCR, and these two distinct structures are required to recognize antigen bound to MHC Class I. CD8 ensures specificity of the TCR–antigen interaction, prolongs the contact between the T cell and the antigen presenting cell, and recruits the tyrosine kinase Lck, which is essential for T cell activation (2).

Tox, Tox2, and TCF1/TCF7 play key roles in T cell development. Tox is also induced by high antigen stimulation during chronic viral infection or cancer, regulating T cell persistence and exhaustion. TCF1/TCF7 preserves the effector function of exhausted T cells during viral infection or cancer. EOMES is a key transcription factor for memory T cells and for full effector differentiation of CD8+ T cells. The dynamic expression of these transcription factors help characterize the extent to which a T cell is exhausted and will respond to antigen stimulation (4-8). Granzyme B is a serine protease expressed by cytotoxic T lymphocytes and natural killer (NK) cells and is a key component of immune responses to pathogens and transformed cells (9).

PD-1 (PDCD1, CD279), TIGIT (VSIG9, VSTM3), TIM-3 (HAVCR2), and LAG3 (CD223) are immune cell co-inhibitory receptors (also known as immune checkpoints) that negatively regulate T cell function and dampen the immune response to pathogens and cancer (10-15). In addition to activated T cells, PD-1 is expressed by activated B cells and monocytes. Following interaction with its ligands, PD-L1 and PD-L2, PD-1 is phosphorylated at ITIM and ITSM motifs leading to recruitment of protein tyrosine phosphatases SHP-1 and SHP-2 and suppression of TCR signaling. TIGIT is expressed at low levels on subsets of T cells and NK cells, and is upregulated at the protein level following activation of these cells. TIGIT marks exhausted T cells in the tumor microenvironment and during human immunodeficiency virus (HIV) infection. TIM-3 is expressed by exhausted T cells in the settings of chronic infection and cancer. Tumor-infiltrating macrophages and dendritic cells also express TIM-3. LAG3 is primarily expressed by activated CD4+ T cells, CD8+ T cells, FoxP3+ T regulatory cells (Tregs), and natural killer (NK) cells. Co-expression of multiple immune checkpoints help characterize the extent to which a T cell is exhausted and will respond to antigen stimulation. Therapeutic blockade of several of these immune

checkpoint receptors is a promising strategy for neoplastic intervention by enabling anti-tumor immune responses (10-15).

Background References

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