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#43049

Human Exhausted CD8+ T Cell IHC Antibody Sampler Kit

Cell Signaling TECHNOLOGY®

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New 01/21

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Isotype/Source
CD3ε (D7A6E™) XP® Rabbit mAb	85061	20 µl	Rabbit IgG
CD8α (D8A8Y) Rabbit mAb	85336	20 µl	Rabbit IgG
Tox/Tox2 (E6I3Q) Rabbit mAb	73758	20 µl	Rabbit IgG
TCF1/TCF7 (C63D9) Rabbit mAb	2203	20 µl	Rabbit IgG
Granzyme B (D6E9W) Rabbit mAb	46890	20 µl	Rabbit IgG
PD-1 (D4W2J) XP® Rabbit mAb	86163	20 µl	Rabbit IgG
TIGIT (E5Y1W) XP® Rabbit mAb	99567	20 µl	Rabbit IgG
TIM-3 (D5D5R™) XP® Rabbit mAb	45208	20 µl	Rabbit IgG
LAG3 (D2G40™) XP® Rabbit mAb	15372	20 µl	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl	Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

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.

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).

Specificity/Sensitivity: Each antibody in the Human Exhausted CD8+ T Cell IHC Antibody Sampler Kit detects endogenous levels of its target human protein. Tox/Tox2 (E6I3Q) Rabbit mAb does not cross-react with Tox3 or Tox4 proteins. TCF1/TCF7 (C63D9) Rabbit mAb does not recognize the dominant negative isoforms of TCF1/TCF7 lacking the amino-terminal β-catenin binding domain and does not cross-react with LEF1. Granzyme B (D6E9W) Rabbit mAb recognizes human Granzyme B protein and is also reactive with mouse Granzyme B; however, this antibody is not suggested for immunohistochemical analysis of mouse tissues. Instead, Granzyme B (E5V2L) Rabbit mAb (Mouse Specific) #44153 is recommended for IHC analysis of mouse tissue samples. Non-specific staining using Granzyme B (D6E9W) Rabbit mAb in the sweat glands has been observed. TIGIT (E5Y1W) XP® Rabbit mAb cross-reacts with an unidentified protein of 42 kDa in some cell extracts.

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.*

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

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(4) Aliahmad, P. et al. (2012) *Curr Opin Immunol* 24, 173-7.
(5) Yao, C. et al. (2019) *Nat Immunol* 20, 890-901.
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(13) Chen, L. and Flies, D.B. (2013) *Nat Rev Immunol* 13, 227-42.
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Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding the carboxy terminus of human CD8α protein, Glu178 of human CD3ε protein, Ala522 of human Tox protein, Pro96 of human TCF1/TCF7 protein, and Ala274 of human PD-1 protein. Monoclonal antibodies are produced by immunizing animals with a recombinant protein specific to human Granzyme B protein, the carboxy terminus of human TIGIT protein, the extracellular domain of human TIM-3 protein, and the amino terminus of human LAG3 protein.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.