

Store at
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#54979

VISTA (D5L5T) XP® Rabbit mAb

Support: +1-978-867-2388 (U.S.)
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orders@cellsignal.comEntrez-Gene ID #74048
UniProt ID #Q9D659

Rev 01/03/18

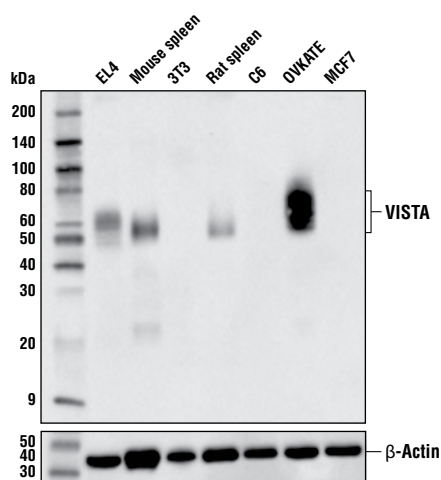
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Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, IF-F Endogenous	H, M, R	45-65 kDa	Rabbit IgG**

Background: VISTA (V-Domain Ig Suppressor of T Cell Activation) is a negative checkpoint control protein that regulates T cell activation and immune responses. VISTA, which contains a single Ig-like V-type domain, a transmembrane domain, and an intracellular domain, has sequence similarity to both the B7 and CD28 family members. Although primarily expressed by myeloid cells, VISTA is also expressed by CD4+, CD8+, and FoxP3+ T-cells. Thus, VISTA is described as both a ligand and a receptor (1-3). Blocking VISTA induces T-cell activation and proliferation, and potentiates disease severity in the EAE model (1). Furthermore, genetic deletion of VISTA in mice leads to spontaneous T-cell activation and chronic inflammation (4,5). In mouse models of cancer, neutralization of VISTA enhances T-cell proliferation and effector function and increases tumor infiltration, suggesting VISTA blockade could be an effective strategy for tumor immunotherapy (6,7).

Specificity/Sensitivity: VISTA (D5L5T) XP® Rabbit mAb recognizes endogenous levels of total VISTA protein. This antibody is not recommended for immunohistochemical analysis of human tissues. Instead, VISTA (D1L2G™) XP® Rabbit mAb #64953 is suggested.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Val291 of mouse VISTA protein.



Western blot analysis of extracts from various cell lines and tissues using VISTA (D5L5T) XP® Rabbit mAb (upper) and β-actin (D6A8) Rabbit mAb #8457 (lower).

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 antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:300†
Unmasking buffer: SignalStain® Citrate Unmasking Solution (10X) #14746	
Antibody diluent: SignalStain® Antibody Diluent #8112	
Detection reagent: SignalStain® Boost (HRP, Rabbit) #8114	
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunohistochemistry (Leica® Bond™)	1:300
Immunofluorescence (IF-F)	1:200
Immunofluorescence (IF-IC)	1:200

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

- (1) Wang, L. et al. (2011) *J Exp Med* 208, 577-92.
- (2) Flies, D.B. et al. (2011) *J Immunol* 187, 1537-41.
- (3) Lines, J.L. et al. (2014) *Cancer Res* 74, 1924-32.
- (4) Wang, L. et al. (2014) *Proc Natl Acad Sci U S A* 111, 14846-51.
- (5) Liu, J. et al. (2015) *Proc Natl Acad Sci U S A* 112, 6682-7.
- (6) Le Mercier, I. et al. (2014) *Cancer Res* 74, 1933-44.
- (7) Lines, J.L. et al. (2014) *Cancer Immunol Res* 2, 510-7.

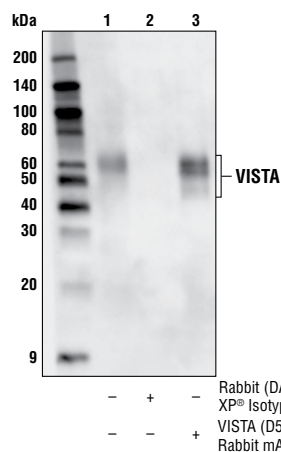
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◀ Immunoprecipitation of VISTA protein from EL4 cell extracts. Lane 1 is 10% input, lane 2 is Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is VISTA (D5L5T) XP® Rabbit mAb. Western blot analysis was performed using VISTA (D5L5T) XP® Rabbit mAb.

-	+	-	Rabbit (DA1E) mAb IgG
-	-	-	XP® Isotype Control
-	-	+	VISTA (D5L5T) XP®
-	-	-	Rabbit mAb

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

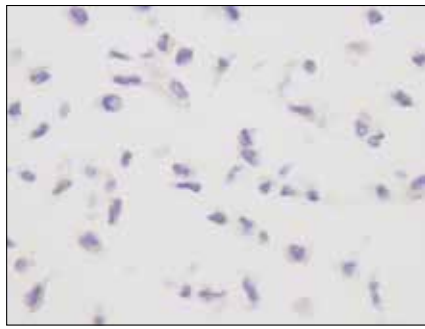
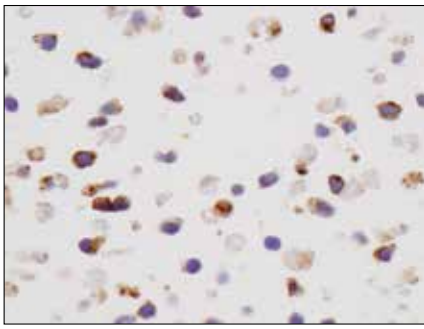
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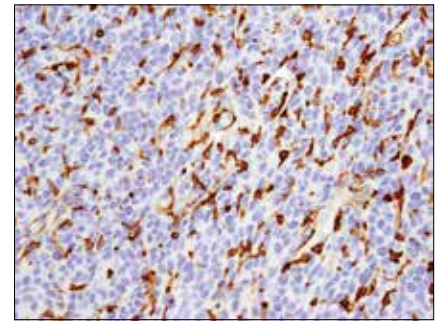
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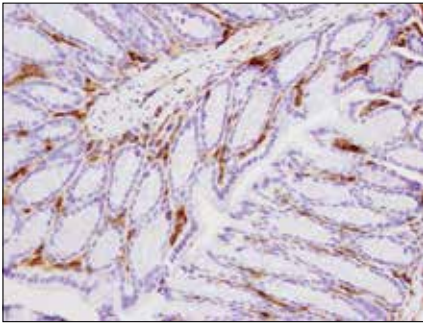
Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine 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.



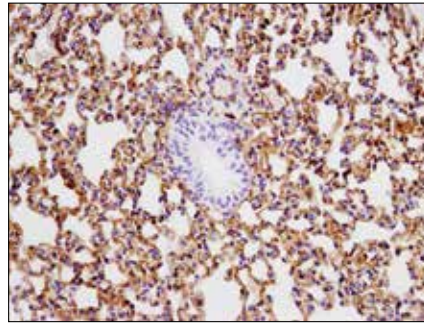
Immunohistochemical analysis of paraffin-embedded EL4 cell pellet (left, positive) or 3T3 cell pellet (right, negative) using VISTA (D5L5T) XP[®] Rabbit mAb.



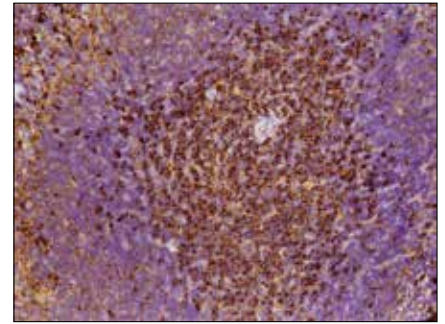
Immunohistochemical analysis of paraffin-embedded mouse CT26 syngeneic tumor using VISTA (D5L5T) XP[®] Rabbit mAb performed on the Leica[®] Bond[™] Rx.



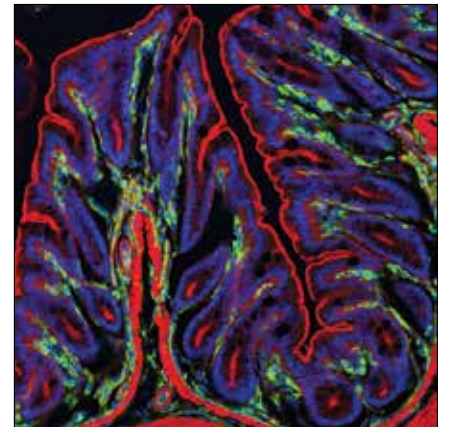
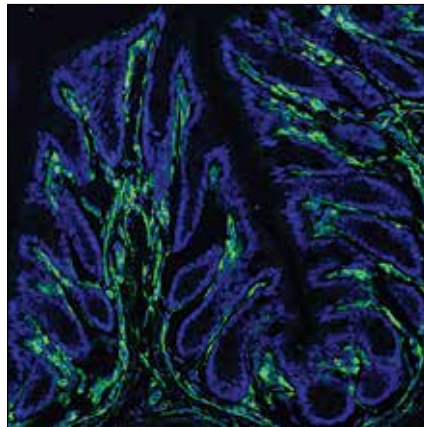
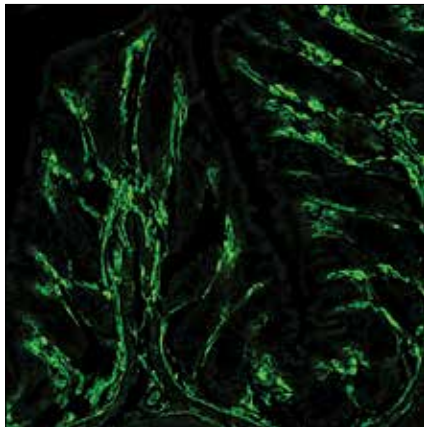
Immunohistochemical analysis of paraffin-embedded mouse colon using VISTA (D5L5T) XP[®] Rabbit mAb.



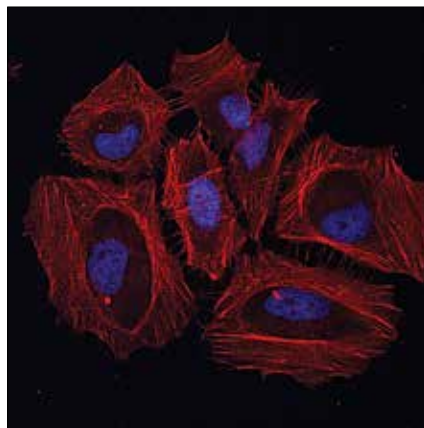
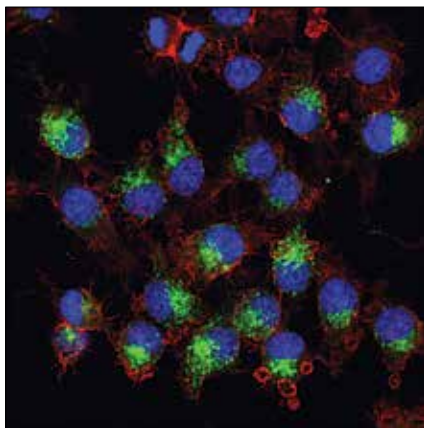
Immunohistochemical analysis of paraffin-embedded mouse lung using VISTA (D5L5T) XP[®] Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded mouse spleen using VISTA (D5L5T) XP[®] Rabbit mAb.



Confocal immunofluorescent analysis of mouse colon using VISTA (D5L5T) XP[®] Rabbit mAb (green). Blue pseudocolor = DRAQ5[®] #4084 (fluorescent DNA dye). Actin filaments were labeled with DyLight[™] 554 Phalloidin #13054 (red).



◀ Confocal immunofluorescent analysis of Raw 264.7 cells (left, positive) and HeLa cells (right, negative) using VISTA (D5L5T) XP[®] Rabbit mAb (green) and a β -Actin Mouse mAb (red). Blue pseudocolor = DRAQ5[®] #4084 (fluorescent DNA dye).

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