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Datasheet for 600-401-378

# V5 Epitope Tag Antibody

## Overview

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| **Description:** | Anti-V5 Epitope Tag (RABBIT) Antibody - 600-401-378 |
| **Item No.:** | 600-401-378 |
| **Size:** | 100 µg |
| **Applications:** | ELISA, WB, IP |
| **Reactivity:** | V5-Tag |
| **Host Species:** | Rabbit |

## Product Details

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| **Background:** | Epitope tags are short peptide sequences that are easily recognized by tag-specific antibodies. Due to their small size, epitope tags do not affect the tagged protein's biochemical properties. Most often sequences encoding the epitope tag are included with target DNA at the time of cloning to produce fusion proteins containing the epitope tag sequence. This allows anti-epitope tag antibodies to serve as universal detection reagents for any tag containing protein produced by recombinant means. This means that anti-epitope tag antibodies are a useful alternative to generating specific antibodies to identify, immunoprecipitate or immunoaffinity purify a recombinant protein. The anti-epitope tag antibody is usually functional in a variety of antibody-dependent experimental procedures. Expression vectors producing epitope tag fusion proteins are available for a variety of host expression systems including bacteria, yeast, insect and mammalian cells. Rockland Immunochemicals produces anti-epitope tag antibodies against many common epitope tags including Myc, GST, GFP, 6X His, MBP, FLAG, HA and V5. Rockland Immunochemicals also produces antibodies to other tags including FITC, Rhodamine (TRITC), DNP and biotin. |
| **Synonyms:** | Rabbit Anti-V5 Epitope Tag Antibody, Rabbit Anti V5 Epitope Tag Antibody, Rabbit Anti-V5 Tag |
| **Host Species:** | Rabbit |
| **Clonality:** | Polyclonal |
| **Format:** | IgG |

## Target Details

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| **Reactivity:** | V5-Tag |

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| **Immunogen Type:** | Conjugated Peptide |
| **Immunogen:** | This antibody was purified from whole rabbit serum prepared by repeated immunizations with V5 epitope tag peptide corresponding to aa 95-108 of the V protein conjugated to KLH using maleimide. |
| **Purity/Specificity:** | This affinity-purified antibody is directed against V5 motif and is useful in determining its presence in various assays. This polyclonal anti-V5-tag antibody detects over-expressed proteins containing the V5 epitope tag. To date this antibody has reacted with all V5 tagged proteins tested so far. In western blotting of bacterial extracts the antibody does not cross-react with endogenous proteins. The antibody recognizes the V5-epitope tag (GKPIPNPLLGLDST) fused to either the carboxy- terminal end of targeted proteins in transfected or transformed cells. Although not yet tested, expect reactivity with recombinant proteins prepared with the V5-epitope tag fused to the amino terminal end as well. |
| **Relevant Links:** | • NCBI - 55775699 |

## Application Details

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| **Tested Applications:** | ELISA, WB |
| **Suggested Applications:** | IP (Based on references) |
| **Application Note:** | Anti-V5 is optimally suited for monitoring expression of V5-tagged fusion proteins. The V5 epitope tag is derived from a small epitope (Pk) present on the P and V proteins of the paramyxovirus of simian virus 5 (SV5). The V5 tag is usually used with all 14 amino acids (GKPIPNPLLGLDST), although it has also been used with a shorter 9 amino acid sequence (IPNPLLGLD). This antibody has been tested by ELISA and western blotting against both the immunizing peptide and V5 containing recombinant proteins. Although not tested, this antibody is likely functional for immunoprecipitation and immunocytochemistry. |
| **Assay Dilutions:** | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below. |
| **ELISA:** | 1:10,000 - 1:60,000 |
| **IHC:** | 1:500 - 1:3,000 |
| **WB:** | 1:5,000 - 1:10,000 |

## Formulation

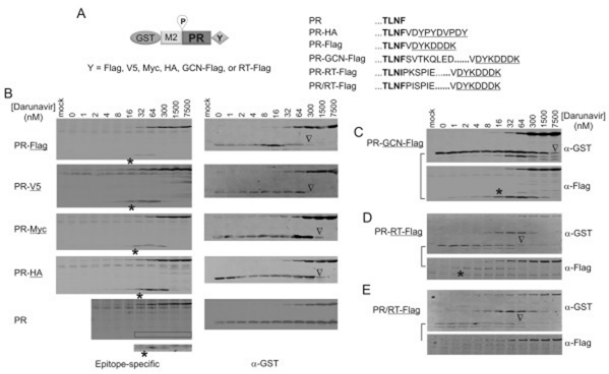
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| **Physical State:** | Liquid (sterile filtered) |
| **Concentration:** | 1.8 mg/mL by UV absorbance at 280 nm |
| **Buffer:** | 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 |
| **Preservative:** | 0.01% (w/v) Sodium Azide |

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| **Stabilizer:** | None |

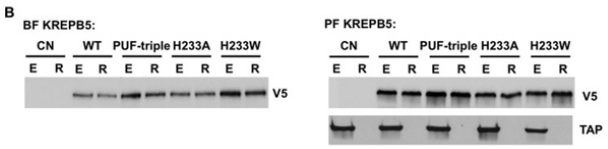
## Shipping & Handling

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| **Shipping Condition:** | Dry Ice |
| **Storage Condition:** | Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use. |
| **Expiration:** | Expiration date is one (1) year from date of receipt. |

## Images

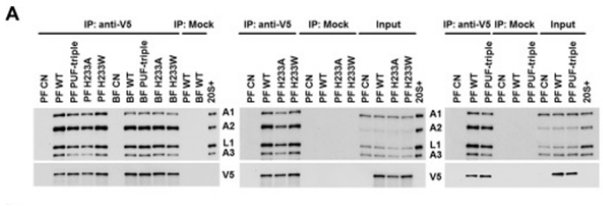



**Western Blot**
Autoprocessing of precursors with different C-terminal tags. All fusion precursors were derived from GST-M2-PRNL-Flag for specific examination of P site cleavage reaction. (A) The sequences at the junction between the PR and the C-terminal tag of some constructs are
listed. (B) Autoprocessing of precursors carrying different small peptide epitopes or no tag were analyzed using a mouse epitope-specific antibody (Flag, Myc, HA), polyclonal rabbit anti-V5, or anti-PR as underlined at left, and GST primary antibodies for dual visualization. Because the signal detected by anti-PR antibody was weak, the boxed region was enhanced to show the mature protease (bottom). Autoprocessing of precursors fused to a GCN4 motif (C) or to reverse transcriptase (RT) in which the native cleavage site between PR and RT remained cleavable (E) or was mutated to uncleavable (D) were also compared. Asterisks indicate self degradation IC50 of the PR-containing product; open triangles indicate the apparent IC50 of the cleavage reaction. The expected products from P site cleavage reaction are connected by a solid line. Fig 5. PMID: 21985091



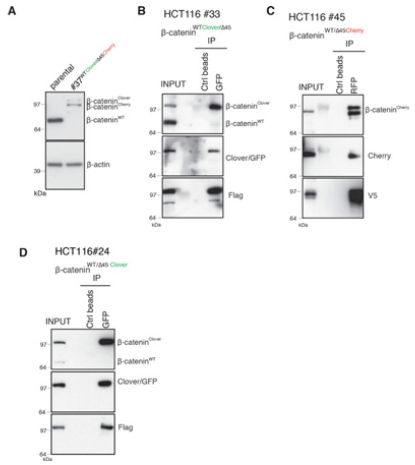

**Western Blot**

B, Western analysis with anti-V5 tag monoclonal antibody showing exclusive expression of V5-tagged mutant or WT KREPB5 proteins from the β-tubulin locus in BF and PF CN cells following repression (R) of the tet-regulatable WT KREPB5 allele for 3 or 4 days, respectively (2 × 106 cells/lane). TAP-tagged WT KREPB5 is detected with PAP antibody in PF cells in which the tet-regulatable allele is expressed (E). Fig 2. PMID: 26304125



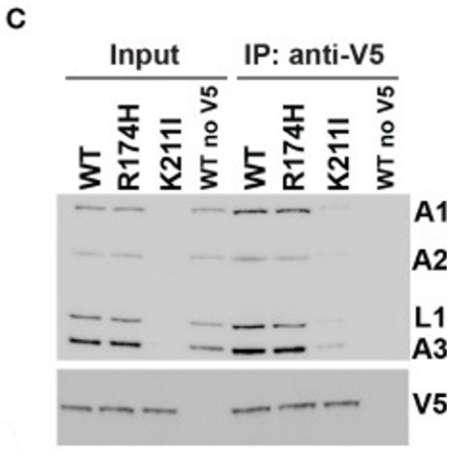

**Immunoprecipitation**

Effects of KREPB5 PUF motif mutations on BF and PF editosomes. A) anti-V5 immunoprecipitation (IP) of exclusively expressed V5-tagged mutant or WT KREPB5 from PF and BF cells in which the tet-regulatable WT KREPB5 allele was repressed for 4 (PF) or 3 (BF) days. Left panel, anti-V5 immunoprecipitates from WT or mutant KREPB5 from PF and BF (6.25 and 12.5% of each immunoprecipitate, respectively) probed with monoclonal antibodies against KREPA1, KREPA2, KREL1, and KREPA3 (A1, A2, L1, and A3) and with anti-V5 tag antibody (V5). No antibody (Mock) and untagged CN cell lysates are controls. Middle and right panels, comparisons of 6.25% of the PF immunoprecipitates and PF controls with 2.5% of cell lysate samples from which the immunoprecipitates were derived. Fig 5. PMID: 26304125

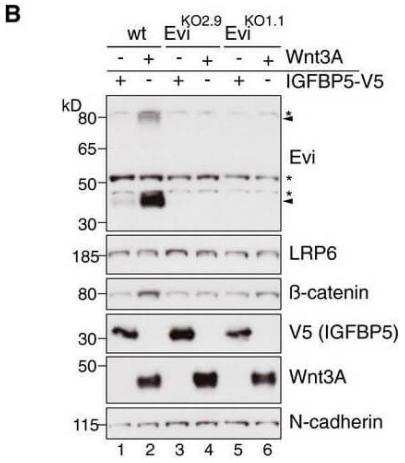



**Western Blot**

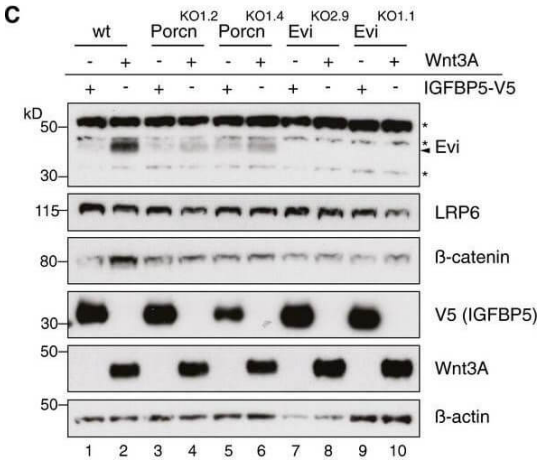
(A) HCT116 β-cateninWTClover/Δ45Cherry (clone #37) cells express comparable amounts of β-catenin to the parental HCT116 WT cells. (B–D) Immunoprecipitation using HCT116 β-cateninWTClover/Δ45 (clone #33 – left), β-cateninWT/Δ45Cherry (clone #45 – middle), and β-cateninWTClover/Δ45 (clone #24 – right) were performed with GFP/Cherry or control beads, followed by Western blotting with the indicated antibodies. Representative results from three independent experiments are shown. SF2. PMID: 26304125



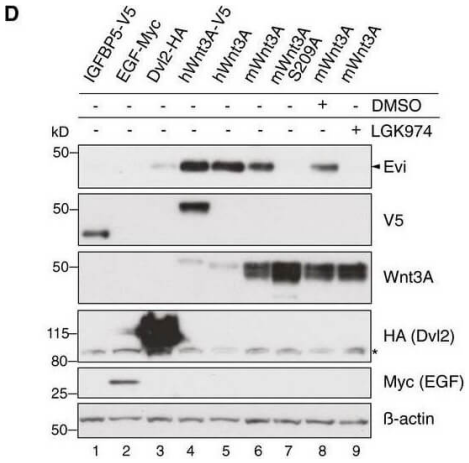

**Western Blot**
(C) BF cells that exclusively expressed V5-tagged WT or mutant B4, or untagged WT B4 control following two days of repression of the regulatable WT B4 allele were used for anti-V5 tag immunoprecipitation of B4-bound protein complexes. Cleared input cell lysates (5%) and anti-V5 immunoprecipitates (10%) were analyzed by Western blotting. Blots were probed with monoclonal antibodies against RECC proteins KREPA1, KREPA2, KREL1 and KREPA3, and anti-V5 antibody. Fig 6. PMID: 38650737



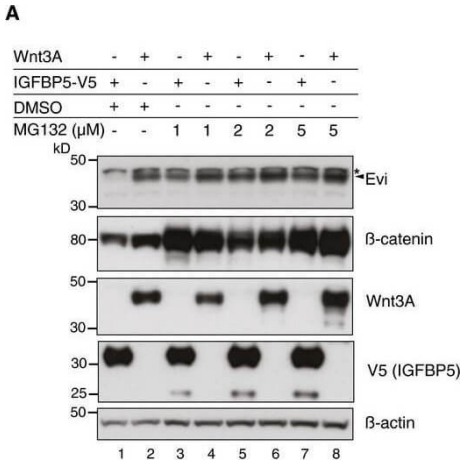

**Western Blot**
Wnt ligand production increases Evi protein levels. Wild-type (wt) or EviKO HEK293T cells were transfected with Wnt3A or IGFBP5-V5 expression plasmids and subjected to Western blot analysis. Specific Evi bands are indicated by arrows and unspecific bands by asterisks. Endogenous Evi is not only detectable as a monomeric form (46 kDa) but also as SDS-resistant dimers (80 kDa). Clonal EviKO HEK293T cells were generated via CRISPR/Cas9 using Evi sgRNA #2 (EviKO2.9) or Evi sgRNA #1 (EviKO1.1; Appendix Fig S2). Figure 1B. Source: EMBO J, PMID: 29378775.



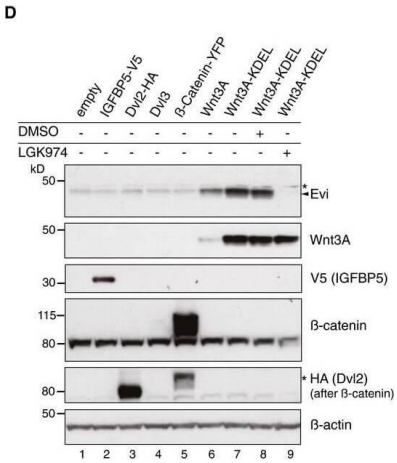

**Western Blot**
Evi stabilization is dependent on Wnt palmitoylation. Western blot analysis of endogenous Evi in wt, PorcnKO, or EviKO HEK293T cells upon overexpression of Wnt3A or IGFBP 5-V5. PorcnKO1.2 and PorcnKO1.4 indicate clone #2 and clone #4 of PorcnKO HEK293T cells generated with Porcn sgRNA1 (Appendix Fig S3). Clonal EviKO HEK293T cells were generated with Evi sgRNA2 (EviKO2.9; clone #9) or Evi sgRNA1 (EviKO1.1; clone #1; Appendix Fig S2). Increase in total β-catenin protein served as control for Wnt pathway activation. Figure 2C. Source: EMBO J, PMID: 29378775.



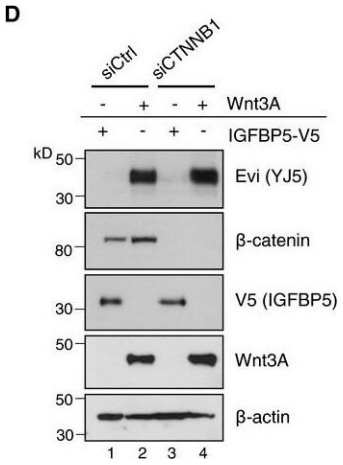

**Western Blot**
Evi stabilization is dependent on Wnt palmitoylation. Western blot analysis of endogenous Evi in HEK293T cells transfected with the indicated overexpression plasmids. When indicated, the cells were additionally treated with 5 µM LGK974 for 48 h. Figure 2D. Source: EMBO J, PMID: 29378775.




**Western Blot**
Evi is ubiquitinated and degraded from the ER by the proteasome.
HEK293T cells were transfected with Wnt3A or IGFBP5-V5 expression plasmids and treated for 24 h with DMSO, MG132, or bortezomib in the indicated concentrations. Figure EV3B. Source: EMBO J, PMID: 29378775.

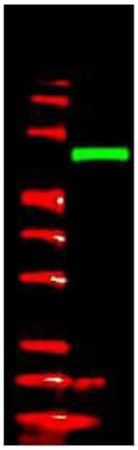



**Western Blot**
Evi poly-ubiquitination is regulated by the presence of Wnt proteins. HEK293T cells were transfected with the indicated plasmids and additionally treated with 5 µM LGK974 for 48 h when indicated. In case of Wnt3A-KDEL, the ER-retaining sequence KDEL was C-terminally fused to Wnt3A. Dvl2-HA, Dvl3, and β-catenin-YFP overexpression was used as negative control to verify that Evi stabilization was not due to downstream activation of Wnt signaling. All Western blots are representative of three independent experiments. β-Actin was used as loading control. Specific Evi bands are marked by arrows and unspecific bands by asterisks. Figure 3D. Source: EMBO J, PMID: 29378775.




**Western Blot**

Evi is not transcriptionally regulated by Wnt. D'Twenty-four hours after reverse transfection with Ctrl or CTNNB1 siRNA, HEK293T cells were transfected with Wnt3A or IGFBP5-V5 expression plasmids and analyzed (D) for the indicated proteins via immunoblotting or (D') for canonical Wnt activity using the TCF-Luciferase Wnt reporter assay. Immunoblotting is representative of three independent experiments, and Wnt reporter activity was calculated as mean from three independent experiments ± s.d. Figure EV1D. Source: EMBO J, PMID: 29378775.



**Western Blot**

Anti-V5 epitope tag polyclonal antibody detects V5-tagged recombinant protein by western blot. This antibody was used at 1.0 µg/ml to detect 0.05 µg (lane 2) of full-length recombinant mouse serum albumin containing the V5 epitope tag at the carboxy end. Comparison to MW markers (lane 1) indicates detection of monomeric V5 tagged albumin. A 4-20% gradient gel was used to separate the protein by SDS-PAGE under non-reducing conditions. The protein was transferred to nitrocellulose using standard methods. After blocking the membrane was probed with the primary antibody overnight at 4° C followed by washes and reaction with a 1:10,000 dilution of IRDye 800 conjugated Gt-a-Rabbit IgG [H&L] (code 611-132-122) for 45 min at room temperature. LICOR's Odyssey® Infrared Imaging System was used to scan and process the image. Other detection systems will yield similar results.

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