Product Datasheet

DYKDDDDK Tag (FLAG) Antibody (orb345397)

Catalog Number	orb345397
Category	Antibodies
Description	Detection of FLAG proteins antibody
Clonality	Polyclonal
Species/Host	Rabbit
Isotype	IgG
Conjugation	Unconjugated
Form/Appearance	Liquid (sterile filtered)
Concentration	1.09
Buffer/Preservatives	Preservative: 0.01% (w/v) Sodium Azide. Stabilizer: None; Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Purity	This affinity purified antibody is directed against the FLAG motif and is useful in determining its presence in various assays. This polyclonal anti-FLAG tag antibody detects over-expressed proteins containing the FLAG epitope tag. In western blotting of bacterial extracts, the antibody does not cross-react with endogenous proteins.
Immunogen	This antibody was purified from whole rabbit serum prepared by repeated immunizations with the Enterokinase Cleavage Site (ECS) peptide DYKDDDDK (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) conjugated to KLH using maleimide. This antibody reacts with FLAG conjugated proteins.
Tested applications	ELISA, KO/KD Validated, WB
Dilution range	ELISA: 1:90,000 - 1:250,000, WB: 1:2,000 - 1:10,000

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Application notes	This antibody is optimally suited for monitoring the expression of FLAG tagged fusion proteins. As such, this antibody can be used to identify fusion proteins containing the FLAG epitope. The antibody recognizes the epitope tag fused to either the amino- or carboxy- termini of targeted proteins. This antibody has been tested by ELISA and western blotting against both the immunizing peptide and FLAGä containing recombinant proteins. Although not tested, this antibody is likely functional for immunoprecipitation, immunocytochemistry, and other immunodetection techniques. The epitope tag peptide sequence was first derived from the 11-amino-acid leader peptide of the gene-10 product from bacteriophage T7. Now the most commonly used hydrophilic octapeptide is DYKDDDDK. Biorbyt's polyclonal antibody to detect FLAG conjugated proteins binds FLAG containing fusion proteins with greater affinity than the widely used monoclonal M1, M2 and M5 clones, and shows greater sensitivity in most assays. Affinity purification of the polyclonal antibody results in very low background levels in assays and low cross-reactivity with other cellular proteins.
Antibody Type	Primary Antibody
Storage	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Dry Ice Shipping	**Please note: This product requires shipment on dry ice. A dry ice surcharge will apply.**
Note	For research use only
Expiration Date	12 months from date of receipt.

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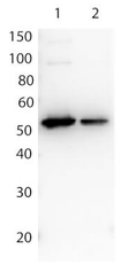

Affinity Purified Antibody to detect FLAG conjugated proteins detects both C terminal linked and N terminal linked FLAG tagged recombinant proteins by western blot. This antibody was used at a dilution of 1:1000 to detect 0.1 µg of recombinant protein containing either the FLAG epitope tag linked at the carboxy (C), Lane 2, or the amino (N), Lane 1, terminus of the recombinant protein. A 4-20% gradient gel was used to resolve the protein by SDS-PAGE. 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 1:40000 dilution of HRP conjugated Gt-a-Rabbit IgG (H&L) MX10 (code orb347654) for 30 min at room temperature.

Biorbyt's antibody to detect FLAG™ conjugated proteins is shown to detect as little as 3 ng of amino-terminal FLAG™ tagged recombinant protein by western blot. This antibody was used at a 1:1000 dilution to detect 3-fold serial dilutions of amino-terminal FLAG™-Bacterial Alkaline Phosphatase (BAP) fusion protein (Sigma P-7582) starting at 1.0 µg of protein as shown in lanes 1-6 respectively. A 4-20% gradient gel was used to separate the protein by SDS-PAGE. The protein was transferred to nitrocellulose using standard methods. After blocking, the membrane was probed with the primary antibody for 1 h at room temperature followed by washes and reaction with a 1:10000 dilution of IRDye® 800 conjugated Gt-a-Rabbit IgG (H&L) for 30 min at room temperature.

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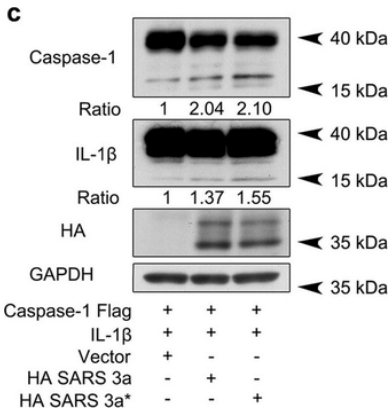
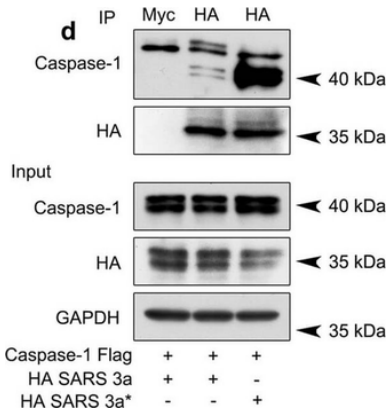


SARS 3a induces NLRP3 inflammasome activation by multiple mechanisms. A) Immunoblot analysis of the pro- and cleaved forms of caspase-1 and IL-1β after reconstitution of inflammasome in HEK 293T cells transfected with SARS 3a with or without NEK7 shRNA. B) Immunoblot analysis of the pro- and cleaved forms of caspase-1 and IL-1β after reconstitution of inflammasome and transfection with SARS 3a or SARS 3a C133A. C) Immunoblot analysis of the pro- and cleaved forms of caspase-1 and IL-1β after co-transfection with caspase-1, IL-1β, and SARS 3a or SARS 3a C133A. D) Immunoprecipitation analysis of interaction between SARS 3a or SARS 3a C133A and caspase-1. All western blot data are representative of two or three independent experiments.



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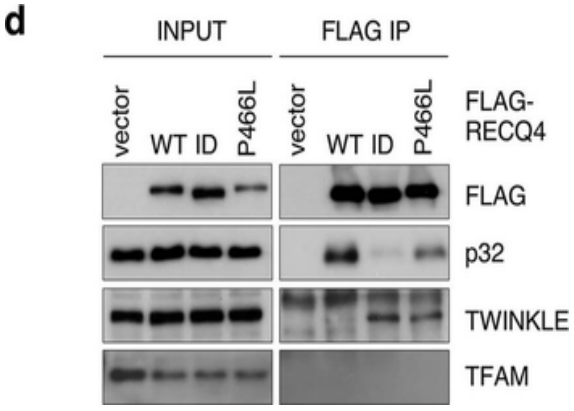

The P466L clinical mutation leads to RECQ4 mitochondrial accumulation. (a) Schematic of human RECQ4 WT, ID and P466L mutant proteins, including the SLD2 (green) and conserved SF2 helicase domains (yellow). (b) Western blot analysis of RECQ4 in WCEs and chromatin-bound (CB) fractions prepared from HEK293 WT or RECQ4 knockdown (KD) HEK293 cells generated by CRISPR technology. Tubulin is used as a loading control. (c) The effects of stable RECQ4 KD shown in (b) and complementation using FLAG-RECQ4 on cell growth as measured by crystal violet cell proliferation assays. Each value represents mean ± standard deviation of 3 independent biological experiments, each with 3 triplicate reactions. (d) Western blot analysis for the presence of WT and mutant FLAG-RECQ4, p32, TWINKLE and TFAM in WCE (left) and immunoprecipitated (IP) with FLAG-RECQ4 complexes (right) in WCEs prepared from stable RECQ4 KD HEK293 cells expressing FLAG-RECQ4 WT, ID or P466L mutant. (e) gDNA levels relative to mtDNA in WCE (left) and MT (right) prepared from stable RECQ4 KD HEK293 cells expressing FLAG-RECQ4 WT, ID or P466L mutant. (f) Western blot analysis of stable RECQ4 KD HEK293 cells expressing FLAG-RECQ4 WT or ID mutant in WCEs and Cyt, MT, and fractions. Tubulin, VDAC1, and lamin A/C are loading and fractionation controls for Cyt, MT, and Nuc fractions, respectively. (g) Western blot analysis of RECQ4 in WCEs and Cyt, MT, and Nuc fractions prepared from stable RECQ4 KD HEK293 cells expressing FLAG-RECQ4 WT or P466L mutant. (h) Representative images showing immunofluorescent staining of FLAG-RECQ4 (green) in stable RECQ4 KD HEK293 cells expressing indicated WT and mutant FLAG-RECQ4 proteins. Mitotracker (red) was used to detect mitochondria, and DAPI (blue) was used to detect nuclei.

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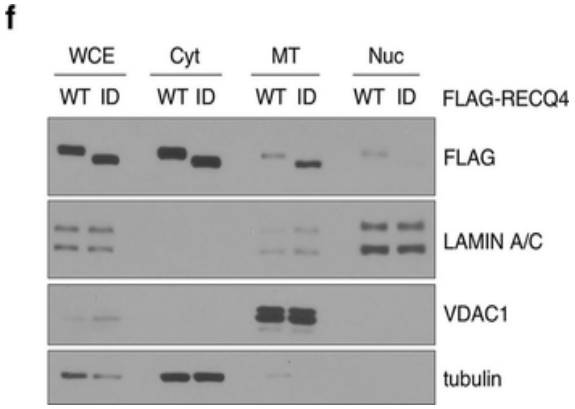

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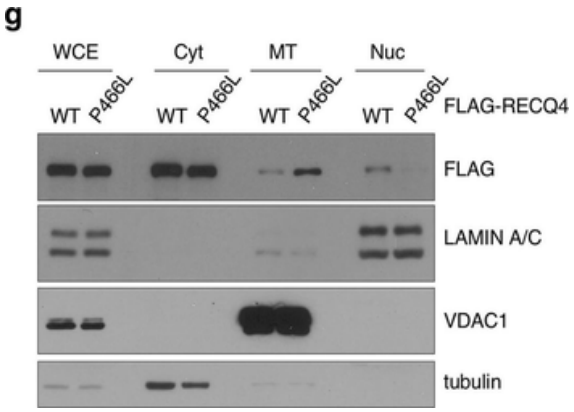

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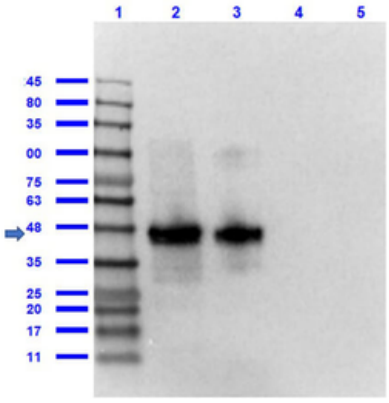

Western Blot of Rabbit anti-FLAG antibody. Lane 1: Opal Prestained Molecular Weight Marker. Lane 2: FLAG Positive Control Lysate (p/n orb535116) (10 µg) [+]. Lane 3: 12 Epitope GST Tagged Lysate (p/n orb535115) (10 µg) [+]. Lane 4: rGST protein (p/n orb345956) (0.1 µg) [-]. Lane 5: MBP protein (0.1 µg) [-]. Primary Antibody: Anti-FLAG at 1.0 µg/ml overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG Peroxidase (p/n orb347654) at 1:70000 at RT for 30 mins. Block: BlockOut Buffer (p/n orb348644). Predicted MW: ~55kDa. Observed MW: ~48kDa. Exposure: 2 sec.

Western Blot of Rabbit anti-FLAG antibody. Marker: Opal Pre-stained ladder. Lane 1: HEK293 lysate (p/n orb348669). Lane 2: HeLa Lysate (p/n orb348668). Lane 3: CHO/K1 Lysate. Lane 4: MDA-MB-231 (p/n orb348700). Lane 5: A431 Lysate (p/n orb348665). Lane 6: Jurkat Lysate (p/n orb348674). Lane 7: NIH/3T3 Lysate (p/n orb348714). Lane 8: E-coli HCP Control (p/n orb342262). Lane 9: FLAG Positive Control Lysate (p/n orb348661). Lane 10: Red Fluorescent Protein (p/n orb345960). Lane 11: Green Fluorescent Protein (p/n orb345957). Lane 12: Glutathione-S-Transferase Protein. Lane 13: Maltose Binding Protein. Load: 10 µg of lysate or 50 ng of purified protein per lane. Primary antibody: FLAG antibody at 1 ug/ml overnight at 4C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n orb347654) at 1:30000 for 60 min at RT. Blocking Buffer: 1% Casein-TTBS for 30 min at RT. Predicted/Observed size: 55 kDa for FLAG.

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