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DDDDK-Tag Rabbit mAb

Catalog No.: AE063 Recombinant 85 Publications

Basic Information

Observed MW

Refer to Figures

Calculated MW

Category

SMab Recombinant Monoclonal Antibody

Applications

WB,IF/ICC,IP,ELISA

Cross-Reactivity

Species independent

CloneNo number

ARC5111-02

Background

FLAG-tag, or FLAG octapeptide, or FLAG epitope, is a polypeptide protein tag that can be added to a protein using recombinant DNA technology, having the sequence motif DYKDDDDK. It has been used for studying proteins in living cells and for protein purification by affinity chromatography. It has been used to separate recombinant, overexpressed protein from wild-type protein expressed by the host organism. It can also be used in the isolation of protein complexes with multiple subunits, because its mild purification procedure tends not to disrupt such complexes. It has been used to obtain proteins of sufficient purity and quality to carry out 3D structure determination by x-ray crystallography. A FLAG-tag can be used in many different assays that require recognition by an antibody. If there is no antibody against a given protein, adding a FLAG-tag to a protein allows the protein to be studied with an antibody against the FLAG sequence. Examples are cellular localization studies by immunofluorescence or detection by SDS PAGE protein electrophoresis and Western blotting.

Recommended Dilutions

WB 1:2000 - 1:6000

IF/ICC 1:50 - 1:200

IP 0.5μg-4μg antibody for

200µg-400µg extracts

of whole cells

ELISA Recommended starting

concentration is 1 µg/mL. Please optimize the concentration based on your specific

assay requirements.

Contact

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Immunogen Information

Gene ID Swiss Prot

Immunogen

Synthetic peptide. This information is considered to be commercially sensitive.

Synonyms

DDDDK; DDDDK tag; DDDDK-tag; DDDDK-Tag

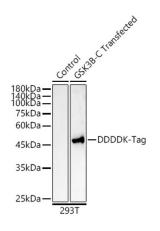
Product Information

SourceIsotypePurificationRabbitIgGAffinity purification

Storage

Store at -20°C. Avoid freeze / thaw cycles.

Buffer: PBS with 0.05% proclin300,0.05% BSA,50% glycerol,pH7.3.



Western blot analysis of lysates from from wild type (WT) and 293T cells transfected with GSK3B-C using DDDDK-Tag Rabbit mAb (AE063) at 1:5000 dilution.

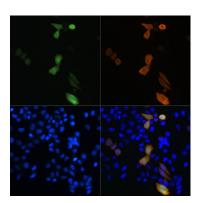
Secondary antibody: HRP-conjugated Goat anti-Rabbit IgG (H+L) (AS014) at 1:10000 dilution.

Lysates/proteins: 25µg per lane.

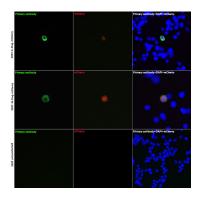
Blocking buffer: 3% nonfat dry milk in TBST.

Detection: ECL Basic Kit (RM00020).

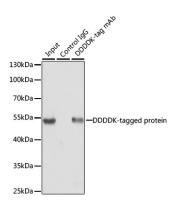
Exposure time: 30s.



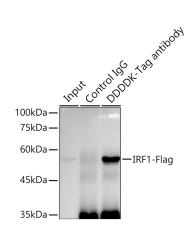
Immunofluorescence analysis of GFP-DDDDK transgenic HeLa cells using DDDDK-Tag Rabbit mAb (AE063). Green: GFP expression. Blue: DAPI for nuclear staining.



Immunofluorescence analysis of 293T-Flag-C and 293T-Flag-N and 293T cells using DDDDK-Tag Rabbit mAb (AE063) at dilution of 1:100 (40x lens). Secondary antibody: Cy3-conjugated Goat anti-Rabbit IgG (H+L) (AS007) at 1:500 dilution. Blue: DAPI for nuclear staining.



Immunoprecipitation of overexpressed DDDDK-tagged protein in 293T cells incubated using DDDDKtag antibody (AE063),Secondary antibody: HRP-conjugated AffiniPure Mouse Anti-Rabbit IgG Light Chain (AS061). A mock served as negative control and over-expressed 293T cell lysate served as positive control.



Immunoprecipitation of IRF1-Flag from 600 μ g extracts of 293T cells transfected with a IRF1 expression vector containing a single N-terminal Flag-Tag was performed using 3 μ g of DDDK-Tag Rabbit mAb (AE063). Rabbit IgG isotype control (AC042) was used to precipitate the Control IgG sample. The IP sample was eluted with 1X reducing Laemmli Buffer. The Input lane represents 10% of the total input. Western blot analysis of immunoprecipitates was conducted using DDDDK-Tag Rabbit mAb (AE063) at a dilution of 1:1000.