Anti-Flag Rabbit Monoclonal Antibody Product Datasheet

Catalog# AYD01-100
Clone# RR690

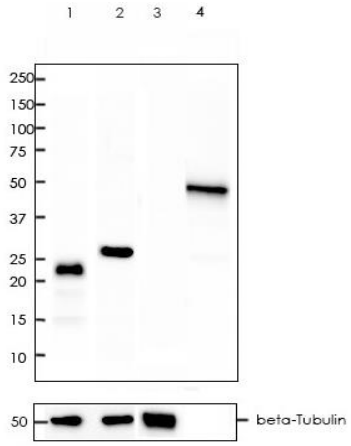
Predicted Molecular Wt: Denpending on customers' target of interest
Species Cross-reactivity: Species independent
Species cross-reactivity determined by WB
Applications: WB IF/ICC FC IP

Purity: ProA affinity purified IgG
Form: Liquid
Swissprot ID: N/A

Background:

Epitope tags are useful for the labeling and detection of proteins using immunoblotting, immunoprecipitation, and immunostaining techniques. Because of their small size, they are unlikely to affect the tagged protein's biochemical properties.

The DYKDDDDK peptide has been used extensively as a general epitope tag in expression vectors. This peptide can be expressed and detected with the protein of interest as an amino-terminal or carboxy-terminal fusion.



Predicted MW: Depend on fusion protein with DYKDDDDK tag
Lane 1: 293 cells lysate transfected with C-terminal DYKDDDDK tagged gene (RR690 at 1:20,000 dilution).
Lane 2: 293 cells lysate transfected with N-terminal DYKDDDDK tagged gene (RR690 at 1:10,000 dilution).
Lane 3: 293 cells lysate without any transfection (RR690 at 1:2,000 dilution).
Lane 4: Multi-tag fusion protein (RR690 at 1:2,000 dilution)
Lane 1/2/3: 3 µg per lane
Lane 4: 20 ng per lane
2nd Ab:
GAR HRP(H+L) 1:5,000
Exposure: 60s

Immunogen:

Synthetic peptide: DYKDDDDK conjugated to KLH.

Storage Buffer:

PBS 59%, Sodium azide 0.01%, Glycerol 40%, BSA 0.05%.

Storage conditions:

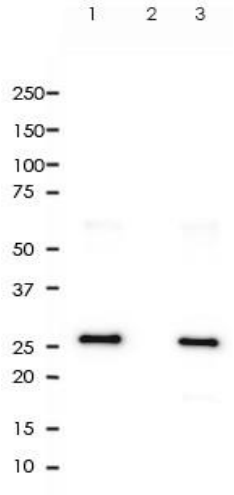
-20°C.

Storage instructions:

Shipped on blue ice. Upon delivery, aliquot, and store at -20°C. Avoid freeze / thaw cycles.

Recommended Dilutions:

WB:	1:10,000 - 1:20,000
IF/ICC:	1:2,000 - 1:10,000
FC:	1:800 - 1:2,000
IP:	1:50

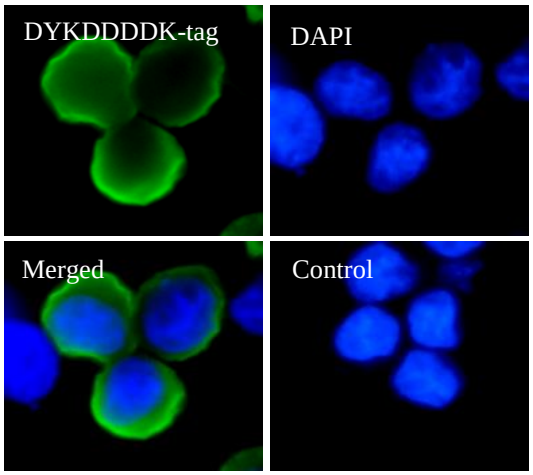


DYKDDDDK tag was immunoprecipitated from 0.1mg of 293 whole cells lysate transfected with N-terminal DYKDDDDK tagged gene with RR690 at 1:50 dilution.
2nd Ab:
GAR HRP for IP 1:500
Lane 1: RR690 IP in 293 whole cell lysate transfected with N-terminal DYKDDDDK tagged gene
Lane 2: PBS instead of RR690 in 293 whole cell lysate transfected with N-terminal DYKDDDDK tagged gene
Lane 3: 293 whole cell lysate transfected with N-terminal DYKDDDDK tagged gene, 2 µg (input)
Exposure: 30s

Background References:

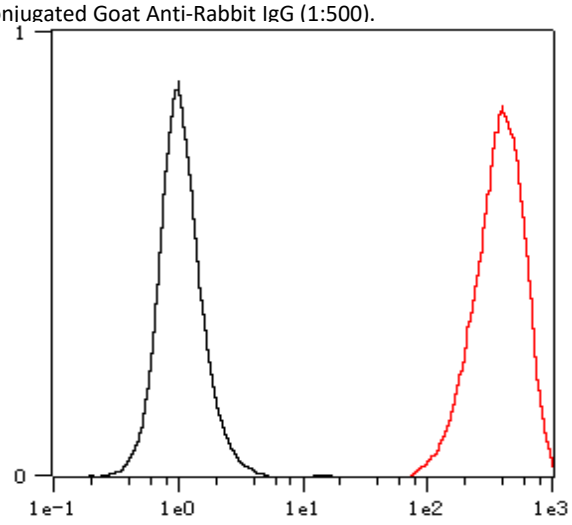
1. Dai X et al. J Proteome Res 12:4167-75 (2013).

2. Németh B et al. FASEB J 30:286-300 (2016).

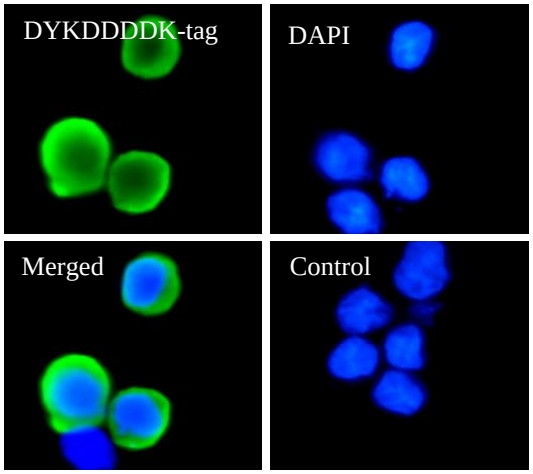


RR690 staining DYKDDDDK tag in 293 cells transfected with N-terminal DYKDDDDK tagged gene by IF/ICC (immunofluorescence/immunocytochemistry). Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 10% goat serum for half an hour at room temperature. Samples were incubated with primary antibody (1:10,000) at 4°C. An Alexa Fluor® 488-conjugated Goat Anti-Rabbit IgG polyclonal was used as the secondary antibody (1:500). DAPI (blue) was used as the nuclear counter stain.

Control: PBS and secondary antibody, An Alexa Fluor® 488-conjugated Goat Anti-Rabbit IgG (1:500).

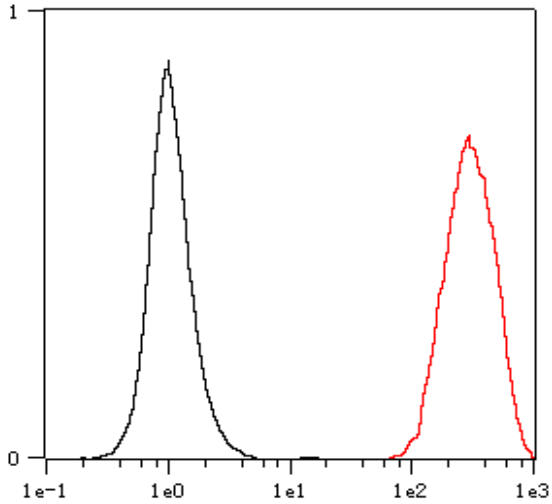


Overlay histogram showing 293 cells transfected with N-terminal DYKDDDDK tagged gene stained with RR690 (Red). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% TritonX-100 for 15 min. The cells were then incubated in the antibody (RR690, 1:2,000 dilution) in 1x PBS/1% BSA for 30 min at room temperature. The secondary antibody used was a Goat Anti-Rabbit Alexa Fluor® 488 (IgG H+L) at 1:2,000 dilution for 20 min at room temperature. Unlabelled sample (Black) was used as a control.



RR690 staining DYKDDDDK tag in 293 cells transfected with C-terminal DYKDDDDK tagged gene by IF/ICC (immunofluorescence/immunocytochemistry). Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 10% goat serum for half an hour at room temperature. Samples were incubated with primary antibody (1:10,000) at 4°C. An Alexa Fluor® 488-conjugated Goat Anti-Rabbit IgG polyclonal was used as the secondary antibody (1:500). DAPI (blue) was used as the nuclear counter stain.

Control: PBS and secondary antibody, An Alexa Fluor® 488-



Overlay histogram showing 293 cells transfected with C-terminal DYKDDDDK tagged gene stained with RR690 (Red). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% TritonX-100 for 15 min. The cells were then incubated in the antibody (RR690, 1:2,000 dilution) in 1x PBS/1% BSA for 30 min at room temperature. The secondary antibody used was a Goat Anti-Rabbit Alexa Fluor® 488 (IgG H+L) at 1:2,000 dilution for 20 min at room temperature. Unlabelled sample (Black) was used as a control.

Product QC'd by:

For research use only. Not for use in diagnostic or therapeutic applications.