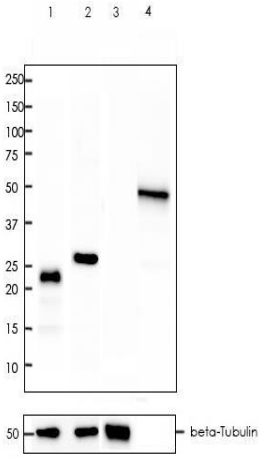
Anti-Flag Rabbit Monoclonal Antibody

Product Datasheet

Catalog# PAR01-100 Clone#RR690

Predicted Molecular Wt: Denpending on customers' target of interest **Purity:** ProA affinity purified IgG
Species Cross-reactivity: Species independent **Form:** Liquid
Species cross-reactivity determined by WB **Swissprot ID:** N/A
Applications: WB IF/ICC FC IP

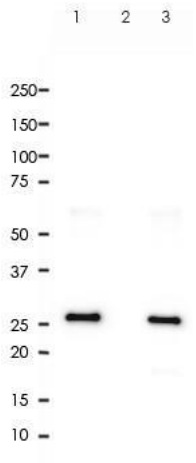
Background:
Epitope tags are useful for the labeling and of proteins using immunoblotting, immunoprecipitation, and immunostaining techniques. Because of their small size, they unlikely to affect the tagged protein ' s properties.
The DYKDDDDK peptide has been used a general epitope tag in expression vectors. peptide can be expressed and detected with protein of interest as an amino-terminal or carboxyterminal 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).
LaneA 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%, BSA0.05%.
Storage conditions:
-20° C.
Storage instructions:
Shipped on blue ice. Upon delivery, aliquot, and storeat -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).**

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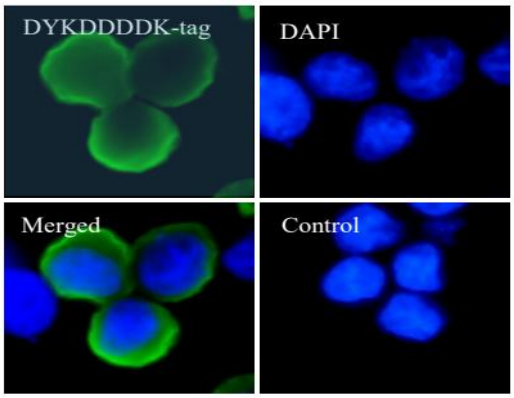
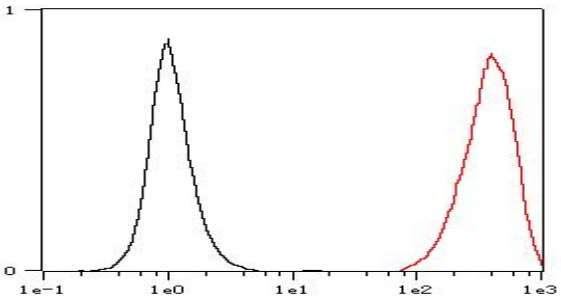
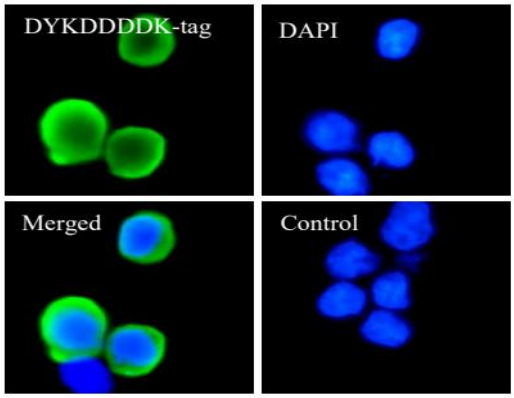


RR690 staining DYKDDDDK tag in 293 cells transfected with Nterminal DYKDDDDK tagged gene by IF/ICC (immunofluorescence/immunocytochemistry). Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 10% goat serum for half an hour at room temperature. Samples were incubated with primary antibody (1:10,000) at 4° C. An Alexa Fluor® 488-conjugated Goat AntiRabbit 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-conjugatedGoatAnti-RabbitIgG(1:500).

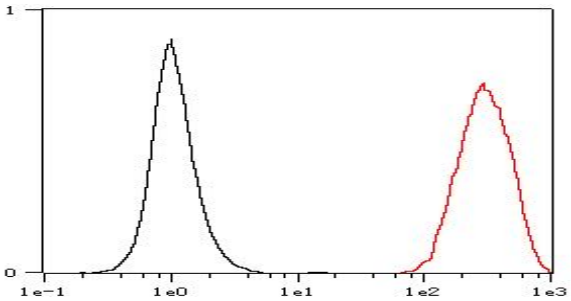


Overlay histogram showing 293 cells transfected with Nterminal DYKDDDDK tagged gene stained with RR690 (Red). The cells were fixed with 4% paraformaldehyde (10min) and then permeabilized with 0.1% TritonX-100 for 15 min. The cells were then incubated in the antibody (RR690, 1:2,000 dilution) in 1x PBS/1% 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 Cterminal 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 AntiRabbit 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-conjugatedGoatAnti-RabbitIgG(1:500).



Overlay histogram showing 293 cells transfected with Cterminal DYKDDDDK tagged gene stained with RR690 (Red).
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Product QC’d by:

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

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