Anti-His Rabbit Monoclonal Antibody

Product Datasheet

Catalog# PAR03-100 Clone#RR689

Predicted Molecular Wt: Depending 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 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.
A variety of plasmids contain DNA that encodes an amino-terminal tag consisting of six histidine (6xHis) residues followed by an extended multiple cloning site. The 6xHis tag on the expressed recombinant proteins allows for efficient coupling to Ni2+ affinity resins and purification by single step chromatography

Immunogen:

Synthetic peptide: HHHHHH (6X His) 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.

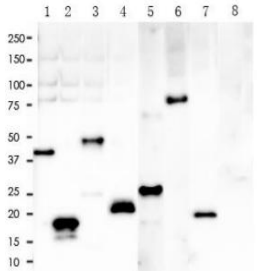
Recommended Dilutions:

WB:	1:10,000 - 1:20,000
IF/ICC:	1:800- 1:2000
FC:	1:200 - 1:1000
IP:	1:50

Background References:

1. Singh AB et al. J Biol Chem 291:5373-84 (2016).

2.Jenkinson EM et al. Nat Genet 48:1185-92 (2016).



This antibody recognizes both C-terminal andN terminal tags.

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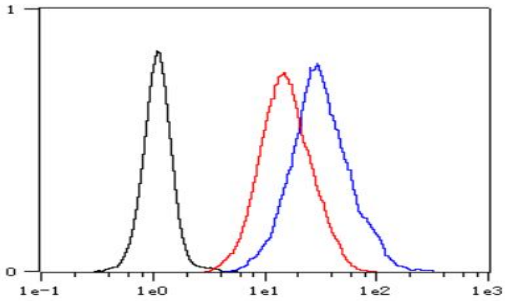
All lanes: Anti-His tag antibody at 1:2,000 dilution
Observed MW: Depend on the fusion protein with His tag

Lane 1: fusion protein with N-terminal 6X His tag
Lane 2: fusion protein with C-terminal 6X His tag
Lane 3: Multi-tag fusion protein containing 6X His tag
Lane 4: 293 cells lysate-transfected with N-terminal 6X His tagged gene
Lane 5: 293 cells lysate-transfected with C-terminal 6X His tagged gene
Lane 6: fusion protein with N-terminal 6X His tag
Lane 7: fusion protein with C-terminal 6X His tag
Lane 8: untransfected 293 cell lysate
Lysate at 20 µg per lane
2nd Ab: GAR HRP(H+L) 1:5,000
Exposure: 60s



Overlay histogram showing 293 cells transfected with N-terminal (Blue) and C-terminal (Red) 6X His tagged gene stained with RR689. The cells were then incubated in the antibody (RR689 , 1:1,000 dilution) in 1x PBS/1% BSA for 30 min at 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.

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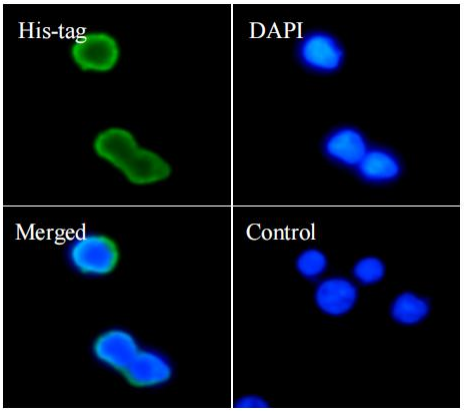
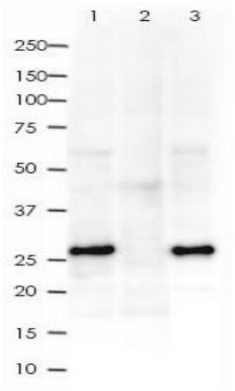
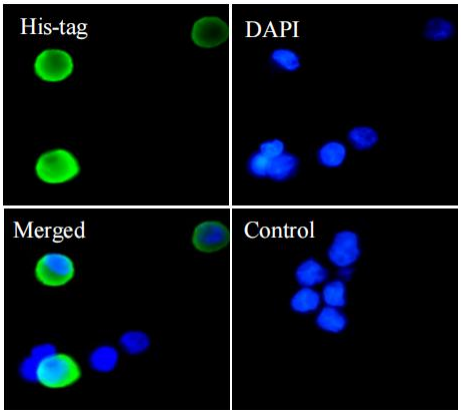


RR689 staining 293 cells trasnfected with N-terminal 6X His tagged gene by IF/ICC (immunofluorescence/ immunocytochemistry).Cells were fixed with paraformaldehyde, permeabilized with 0.1%Triton X-100 and blocked with 10% goat serum for half an hour at room temperature. Sampleswere incubated with primary 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)

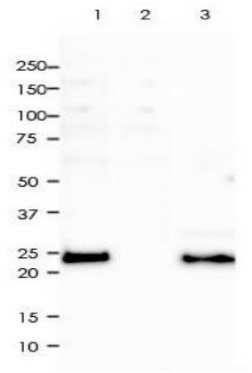


His tag was immunoprecipitated from 0.05mg of 293 whole cells lysate transfected with N-terminal 6X His tagged gene with RR689 at 1:50 dilution.
2nd Ab: GAR HRP for IP 1:500
Lane 1: RR689 IP in 293 whole cell lysate transfected with N terminal 6X His tagged gene
Lane 2: PBS instead of RR689 in 293 whole cell lysate transfected with N-terminal 6X His tagged gene
Lane 3: 293 whole cell lysate transfected with N-terminal 6X His tagged gene, 10 µg (input)
Exposure: 20s



RR689 staining 293 cells trasnfected with C-terminal 6X Histagged 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 (1:800) 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).



His tag was immunoprecipitated from 0.2mg of 293 whole cells lysate transfected with C-terminal 6X His tagged gene with RR689 at 1:50 dilution.
2nd Ab:
GAR HRP for IP 1:500
Lane 1: RR689 IP in 293 whole cells lysate transfected with C terminal 6X His tagged gene
Lane 2: PBS instead of RR689 in 293 whole cells lysate transfected with C-terminal 6X His tagged gene
Lane 3: 293 whole cells lysate transfected with C-terminal 6X His tagged gene, 10 µg (input)
Exposure: 120s

Product QC’dby:

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

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