Anti-$(HIS)_6$ TAG
Epitope Tag Antibody – Unconjugated (Azide and BSA Free)
Mouse Monoclonal Antibody Clone 13/45/31-2

Product Information

Catalog No.:	**DIA-910-1MG-ABF (1 mg)**
	DIA-910-200-ABF (200 µg)
Clone:	13/45/31-2
Concentration:	1.0 mg/ml
Isotype:	Mouse IgG1, kappa
Specificity:	Recognizes N-terminal, C-terminal or internal HIS-tagged fusion proteins with at least 6 histidine residues
Immunogen:	Recombinant (HIS)6-p53 protein
Species Reactivity:	Not Applicable
Physical State:	Liquid
Positive Control:	$(HIS)_6$-protein (not included)

Presentation:	In PBS pH 7.4. Antibody purified from cell culture supernatant
Sensitivity:	WB: 1ng HIS-tagged protein
Affinity:	3x 10-10M (Biacore™ Analysis)
Applications	**Dilution**
ELISA	0.1 - 0.4 µg/ml
Flow Cytometry	5 - 10 µl/10^6 cells
IHC-frozen	1 - 4 µg/ml
Immunofluorescence	1 - 4 µg/ml
Immunoprecipitation	4 µg/ml
Western Blot	1 - 4 µg/ml

General recommendations, optimal dilutions should be determined by the end user by titration test

Reactivity

The mouse monoclonal anti-$(His)_6$-tag antibody, clone 13/45/31-2 (H. Zentgraf/DKFZ Heidelberg, Germany), specifically detects any kind of histidine-tagged proteins in cells and complex cellular lysates. This monoclonal antibody specifically reacts with recombinant proteins containing an epitope of at least 6 histidine residues, located at the N-terminus, C-terminus or internally. A higher number of histidine residues leads to an increased binding affinity of the antibody, e.g. an expression construct with 10 histidine residues increases the affinity about 10- to 20-fold. Additional flanking amino acids are not required for antibody binding, therefore enabling the choice of many different expression vectors on the only condition that the TAG-epitope is sterically available.

Background

Recombinant proteins are utilized for various purposes in molecular biology. Many prokaryotic expression vectors have been established enabling synthesis of the protein of interest as a fusion with a peptide, thus facilitating purification. Expression of recombinant proteins in E. coli as a fusion protein that includes histidine residues (His) as tag is one of the most popular methods, because histidine tagged proteins have useful attributes. The affinity of the histidine-tag motif to Ni2+ by chelation is strong and selective enough to enable purification of the protein to homogeneity by affinity chromatography on a Ni2+-NTA adsorbant and the resulting protein can be selectively detected using antibodies such as clone 13/45/31-2.

References

The Antibody clone 13/45/31-2 /13/45/31 is with **more than 100 citations** one of the most cited His-Tag Antibodies world wide. For a comprehensive list of citations visit:
https://www.dianova.com/his-references/

Initial Publication:

1. Zentgraf H, Frey M, Schwinn S, Tessmer C, Willemann B, Samstag Y, Velhagen I. Detection of histidine-tagged fusion proteins by using a high-specific mouse monoclonal anti-histidine tag antibody. Nucleic Acids Res.16,3347-8, 1995.

Storage and Stability

Do not freeze. Product is stable for 1 year from the date of shipment when stored at 2-8 °C. Does not contain stabilizers and preservatives. Please handle under sterile contditons to avoid contamination.

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Buffer recommendations for Western Blotting and Immunofluorescence

Incubate the antibody in the presence of BSA!
For antibody incubation the total protein concentration of the dilution buffer should not be less than 0.2mg/ml!

The binding of the anti-His-Tag antibody to a (His)6 motif is a cooperative process which depends on the neighbouring fusion protein. For enabling quantitative formation of a stable antigen-antibody complex it is recommended to use high antibody concentrations (1 to 4 µg/ml**).**

Recommended blocking buffer: PBS, 0.1% Tween, 0.1% Triton (PBST) + 5% BSA.
Recommended dilution buffer: PBST + 5% BSA.

Western Blot Detection

After electrophoresis of protein extract (8 µg) and Western Blot the anti-histidine-tag antibody diluted 1:500 allows the detection of 1 ng His-Tag protein. The antibody diluted 1:5.000 allows the detection of 3-6 ng His-Tag protein with alkaline phosphatase and BCIP/NBT detection system. For lower detection limits or more sensitive Western Blotting detection systems higher dilution are possible. If problems are encountered using standard Western blotting protocols we recommend trying the following modifications:

- Prolong incubation with anti-His-Tag antibody up to 14 h at 4°C. The incubation buffer should contain 0.02% NaN3.
- Use PVDF membranes for Western or Dot blotting which can be superior to nitrocellulose in presenting the (His)6 motif.
- For Dot or Western blotting residual nickel should be removed by incubating the membrane as follows (steps a - d):
  a) Protein transfer to PVDF membrane
  b) Incubation of the membrane for 1 h in PBS with 2% BSA
  c) Incubation for 30 min in PBS with 2 mg/ml BSA and 100 mM EDTA
  d) Wash 2 x with Western blot buffer

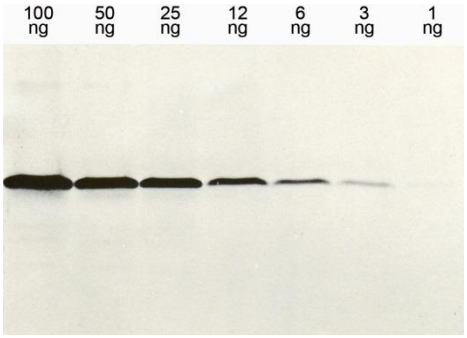

Fig.1 Detection of (HIS)6-p53 Hela cell lysates (Transfection with HIS-tagged protein vector, SDS-PAGE and western blotting according to standard procedures). Sensitivity: Specific detection of 1ng protein in 8µg total proteins at a 1:100 antibody dilution / Antibody dilutions up to 1:10.000 for less sensitive detections (ca. 6ng HIS-tagged protein).

General Western Blot Recommendations

For a general western Blot recommendations visit
https://www.dianova.com/western-blot-faq/

Immunofluorescent Detection

For immunofluorescence stainings dilutions of 1/250 – 1/10.00 are recommended

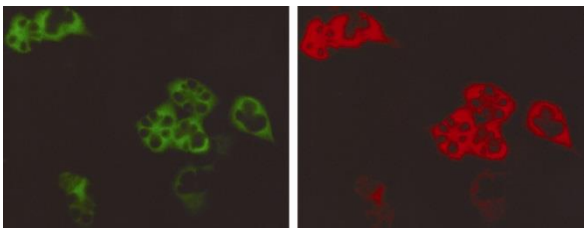

Fig.2 Immunofluorescence detection of (HIS)6-GFP in Hela-cells transfected with expression plasmid pCMV-His6-GFP-APKD1-5a. LEFT: GFP autofluorescence; RIGHT: 1:500 anti-HIS #DIA-900 (in red) and Cy3-labeled goat anti-mouse secondary antibody.

Safety Notes

The material does not contain preservatives or stabilizers. Please handle all reagents with proper care.

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