

13/45/31-2

Anti-(HIS)₆ TAG **Epitope Tag Antibody – Biotin-conjugated** Mouse Monoclonal Antibody Clone 13/45/31-2

Product Information

Clone:

Specificity:

Catalog No.: DIA-900-BIOT (100µg) Presentation: In PBS with 6% BSA, 0.05% NaN3,

pH 7.4. Antibody purified from

cell culture supernatant

Concentration: Sensitivity: WB: up to 1ng HIS-tagged protein 1.0 mg/ml

3x 10-10M (Biacore™ Analysis) Affinity: Isotype: Mouse IgG1, kappa

> **Applications** Dilution Recognizes N-terminal, Cterminal or internal HIS-tagged

ELISA $0.1 - 0.4 \, \mu g/ml$ fusion proteins with at least 6 Flow Cytometry 5 - 10 μl/10⁶ cells histidine residues 1 - 4 µg/ml **IHC-frozen**

Immunogen: Recombinant (HIS)6-p53 protein Immunofluorescence 1 - 4 µg/ml Not Applicable **Species Reactivity:** Immunoprecipitation 4 µg/ml

Physical State: Liquid Western Blot 1 - 4 µg/ml **B/P-ratio:** ~3-5

General recommendations, optimal dilutions should be determined

by the end user by titration

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:

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 $^{\circ}\text{C}$

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



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Data Sheet Version:10 - (03.12.2018) Page: 2 / 2

Buffer recommendations

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 neighboring fusion protein. For enabling quantitative formation of a stable antigen-antibody complex it is recommended to use high antibody concentrations (1 to 4 μ g/ml).

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

Recommended dilution buffer: PBST + 5% BSA.

Instructions for use

Following dilutions are recommended:

Applications Dilution 0.1 - 0.4 μg/ml **ELISA** Flow Cytometry $5 - 10 \,\mu l / 10^6 \, cells$ **IHC-frozen** $1 - 4 \mu g/ml$ Immunofluorescence 1 - 4 μg/ml Immunoprecipitation 4 µg/ml

1 - 4 μg/ml Optimal dilutions should be determined by the end user by titration.

Safety Notes

Western Blot

The material contains 0.05% sodium azide as preservative. Although the quantity of azide is very small, appropriate care should be taken when handling this material. Avoid skin and eye contact, inhalation, and ingestion. For more information please refer to the Material Safety Datasheet (MSDS).

