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Product no AS20 4483

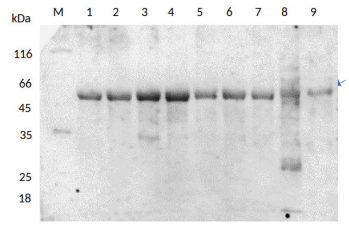
Anti-Tubulin alpha chain (monoclonal antibody)

Product information

Immunogen	Fraction of tubulin purified from porcine brain by two cycles of polymerization - depolymerization, UniProt: Q71U36
Host	Mouse
Clonality	Monoclonal
Subclass/isotype	IgG1
Purity	Immunoglobulin Protein A purified in PBS. Contain 15 mM sodium azide.
Format	Liquid
Quantity	100 µg
Storage	Store at 4°C; Do not freeze. Do not exceed exipry date is provided on the tube. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
Additional information	Metal induced stress affected the expression of tubulin, and that therefore, this protein cannot be used as a loading control under that type of conditions, data in application example,

Application information

Recommended dilution	1 : 500 (ICC), 1-4 µg/ml /FlowCyt), 1-4 µg/ml (WB)
Expected | apparent MW	51 kDa
Confirmed reactivity	*Arabidopsis thaliana, Chlorella vulgaris, Eisenia*, human, mouse, *Nicotiana tabacum*, *Paramecium* sp. , pig, turkey, yeast
Predicted reactivity	Plants Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	This antibody is recognizing defined epitope (amino acid 65-97) on N-terminal structural domain of alpha tubulin. Recommended secondary antibody, goat anti-mouse IgG1, HRP conjugated AS16 3715
Selected references	Liu et al. (2022) Identification of positive and negative regulators of antiviral RNA interference in Arabidopsis thaliana. Nat Commun. 2022 May 30;13(1):2994. doi: 10.1038/s41467-022-30771-0. PMID: 35637208; PMCID: PMC9151786.



Samples:

1 - 14 µg of *Nicotiana tabacum*, L. whole leaf extract of adult control plant (untreated)
2 - 14 µg of *Nicotiana tabacum*, L. whole leaf extract of adult plant after treatment with 100 µM AgNP-PVP
3 - 14 µg of *Nicotiana tabacum*, L. whole leaf extract of adult plant after treatment with 100 µM AgNP-CTAB
4 - 14 µg of *Nicotiana tabacum*, L. whole leaf extract of adult plant after treatment with 100 µM AgNO3
5 - 14 µg of *Nicotiana tabacum*, L. whole leaf extract of adult plant after treatment with 100 µM AgNP-PVP+cys
6 - 14 µg of *Nicotiana tabacum*, L. whole leaf extract of adult plant after treatment with 100 µM AgNP-CTAB+cys
7 - 14 µg of *Nicotiana tabacum*, L. whole leaf extract of adult plant after treatment with 100 µM AgNO3+cys
8 - 14 µg of *Chlorella vulgaris* extract- control (untreated)
9 – 7 µg of *Arabidopsis thaliana* seedlings extract- control (untreated)

14 µg/well of total protein extracted freshly from 0.05 g of lyophilized samples as specified above, following the Phenol extraction protocol 1 with extraction buffer containing Trizma base (500 mM), Ethylenediaminetetraacetic acid (EDTA) (50 mM), sucrose (700 mM) and Potassium chloride (KCl) (100 Mm) with addition of phenylmethylsulfonyl fluoride (PMSF) (1mM) and 2% -mercaptoethanol. After short incubation on 4°C with

agitation, phenol was added. The phenol (supernatant) phase containing proteins, was collected after centrifugation and equal volume of extraction buffer was added. After centrifugation, supernatant phase was collected and 4 volumes of 0.1 M ammonium acetate (with 10% methanol) was added, and proteins were precipitated ON/-20°C. The next day, protein pellets were washed 3 times in ammonium acetate with rounds of centrifugations in between, and finally in aceton. Protein pellet was lastly resuspended in Isoelectric focusing buffer (IEF) containing 9 M urea, 4% CHAPS, 20 mM DTT, 1.2% Ampholytes pH 3 to 10. Protein concentrations was measured with modified Bradford method1 and denatured with Laemmli sample buffer2 at 95°C for 5 min. Total proteins were separated on 12% SDS-PAGE and blotted 1h to nitrocellulose (pore size of 0.2 µm), using wet transfer. Blot was blocked with 2% milk in PBS-T, 1h/RT. Blot was incubated in the primary antibody at a dilution of 1: 1 000 for 1h/RT with agitation in a solution of 2% milk in PBS-T and then ON/4°C. The antibody solution was decanted and the blot was then washed 3 times for 10 min in 2% milk in PBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (goat anti-Mouse IgG1 HRP conjugated AS16 3715) diluted to 1:8000 in 2% milk in PBS-T for 1h/RT with agitation. The blot was washed twice for 10 min in PBS-T developed for 5 min with AgriseraECLSuperBright (AS16 ECL-S). Exposure time was 12 min.

Metal induced stress affected the expression of tubulin, and that therefore, this protein cannot be used as a loading control under that type of conditions

Courtesy of MSc, Karla Košpić, University of Zagreb, Faculty of Science Department of Biology, Croatia