



Catalog No. MB-001-B

Anti-Pan Ubiquitin Monobody (Biotin)

An artificial binding protein that recognizes ubiquitin monomer, mono-ubiquitinated proteins, and poly-ubiquitinated proteins, which is biotinylated and compatible to avidin and streptavidin reagents for detection.

Product Details

Size	50 µg
Product type	Monobody
Theoretical MW	14.3 kDa (not containing biotin moiety)
Fusion tag	His-tag
Conjugate	Biotin
Specificity/target	Ubiquitin monomer, mono/poly (K6, K11, K27, K29, K33, K48, K63, M1)-ubiquitinated proteins.
Species reactivity	All species expected
Affinity (Kd)	0.88 nM
Tested applications	IP, IHC, ICC/IF, WB, ELISA
Purification	Immobilized Metal Chromatography
Form	Liquid
Concentration	0.5 mg/mL
Storage buffer	25 mM Na-Phosphate (pH8.0), 250 mM NaCl, 50% Glycerol, 0.05% ProClin 300
Storage condition	For continuous use, store at -20°C for up to six months. For long-term storage, freeze at -80°C. Avoid repeated freeze/thaw cycles.

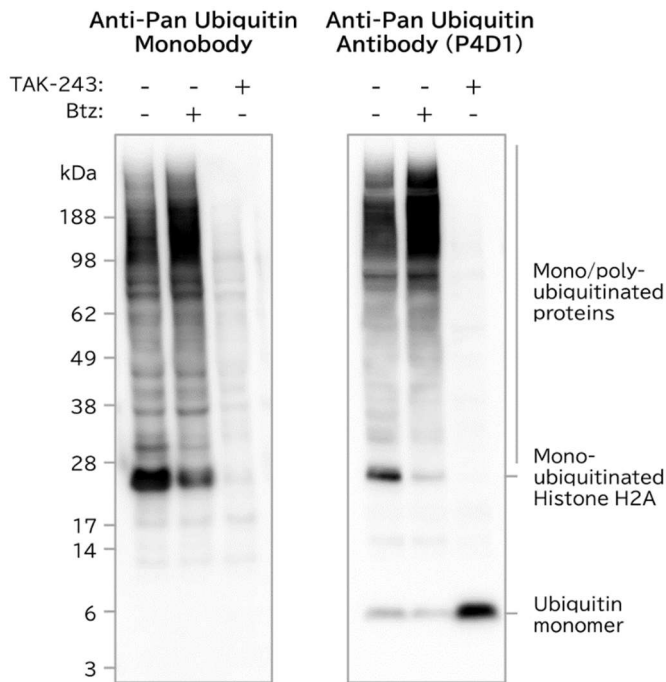
Application Notes

Application	Recommended dilution
WB	1:1,000-1:10,000 (see comments)
ICC/IF	1:500-1:5,000 (see comments)
Comments	This monobody has been confirmed to recognize ubiquitin monomer in ELISA and Immunoprecipitation (IP) whereas not in Western Blotting (WB) and to recognize mono-ubiquitinated proteins and poly-ubiquitinated proteins in IP and WB. It is recommended that the monobody be titrated for optimal performance in individual systems.
Caution	Do not use skim milk for blocking and antibody dilution in assay with avidin reagent, as skim milk contains biotin which would interfere with avidin-biotin detection systems.



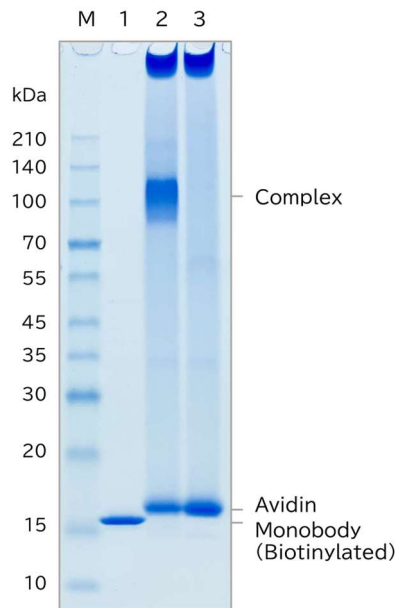


Application Data

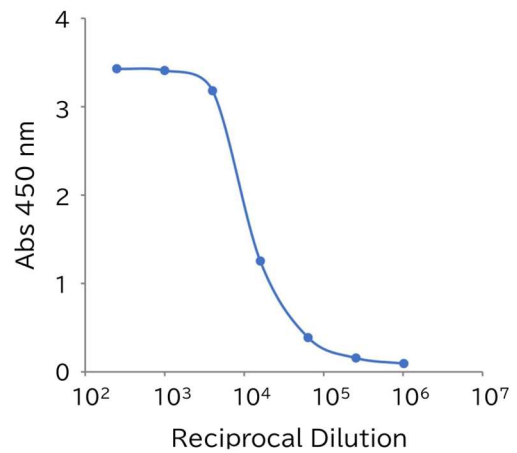


Detection of ubiquitination by Western blot analysis. HCT-116 cells were treated with TAK-243 or Btz for 3 hours. Lysates were then immunoblotted with Anti-Pan Ubiquitin Monobody (left) and Anti-Pan Ubiquitin Antibody (mouse IgG, clone P4D1) (right), the former visualized by chemiluminescence with streptavidin-HRP and the latter with anti-mouse IgG-HRP. Anti-Pan Ubiquitin Monobody was more reactive to mono-ubiquitinated histone H2A and low molecular size-ubiquitinated proteins than the antibody, clone P4D1, whereas the monobody did not recognize ubiquitin monomer. This result appears to be largely consistent with that in immunoprecipitation performed in another experiment in which the Anti-Pan Ubiquitin Monobody showed little bias against various types of ubiquitination.

Validation Data



Purity and biotinylation efficiency of Anti-Pan Ubiquitin Monobody. 1) 1 µg of the monobody, 2) 1 µg of the monobody incubated with 5 µg of Avidin and 3) 5 µg of Avidin were analyzed by SDS-PAGE with CBB staining.



Reactivity of Anti-Pan Ubiquitin Monobody to ubiquitin monomer. Dilution series of the monobody were tested in ELISA using an immunoassay plate coated with 1 µg/well of recombinant human ubiquitin monomer. NeutrAvidin-HRP was used for color development.

