

Application Notes

Catalog No. MB-001-B

Anti-Pan Ubiquitin Monobody (Biotin)

May. 29, 2025

This product is a biotinylated artificial binding protein that specifically binds to ubiquitin monomers, as well as mono- and polyubiquitinated proteins, regardless of ubiquitin chain length or linkage type. It works with secondary detection reagents such as avidin, streptavidin, and anti-6×His antibody-based reagents.

Product Details	
Size	50 μg
Product Type	Monobody
Theoretical MW	14.3 kDa (excluding biotin moiety)
Fusion Tag	His-tag
Conjugate	Biotin
Specificity/Target	Ubiquitin monomer; mono-/poly-ubiquitinated proteins (K6, K11, K27, K29, K33, K48, K63, M1)
Species Reactivity	Expected to react with all species
Affinity (<i>Kd</i>)	0.88 nM
Applications	IP, IHC, ICC/IF, WB, ELISA
Purification Method	Immobilized Metal Affinity Chromatography (IMAC)
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 Conditions	Store at -20°C for up to 6 months for frequent use. For long-term storage, keep at -80°C. Avoid repeated freeze-thaw cycles.

Application	Recommended starting dilutions are 1:1,000-1:10,000 for WB and 1:500-1:5,000 for ICC/IF.
Additional Info.	 This product binds to ubiquitin monomer in ELISA and immunoprecipitation (IP), but not in Western blot (WB). It binds to mono- and poly-ubiquitinated proteins in both IP and WB. Titration is recommended to determine optimal conditions for specific experimental systems.
Important Info.	Reliable Western blot signals have been observed only when BSA is used for both blocking and antibody dilution, regardless of the secondary detection reagent. Skim milk and polymer-based blockers are not recommended. Compatibility with avidin- and streptavidin-based detection reagents may vary by product. Streptavidin-HRP (Product No. SA10001, Thermo Fisher Scientific Inc.) has shown strong compatibility with this product.



Application Data

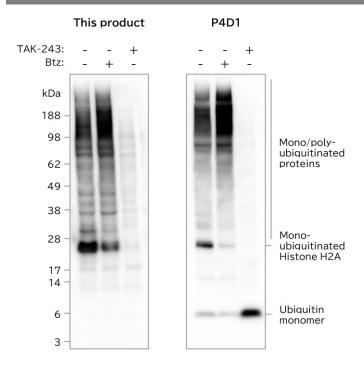


Figure 1. Comparison of Detection Sensitivity Between This Product and P4D1 in Western Blotting. HCT-116 cells were treated with TAK-243 (a selective inhibitor of the ubiquitinactivating enzyme) or bortezomib (Btz; a selective proteasome inhibitor) for 3 hours. Cell lysates were analyzed by SDS-PAGE and Western blotting. Blots were probed with this product (Anti-Pan Ubiquitin Monobody, Biotin; labeled as "This product" in the figure) and a conventional anti-ubiquitin antibody (clone P4D1; labeled as "P4D1"), both at 1:1,000. Detection was by chemiluminescence (ECL) using streptavidin-HRP and anti-mouse IgG-HRP, each at 1:10,000. Exposure times were 3 seconds for this product and 30 seconds for P4D1. This product showed enhanced sensitivity toward mono-ubiquitinated histone H2A and low molecular weight ubiquitinated proteins, but did not detect free ubiquitin monomers.

Validation Data

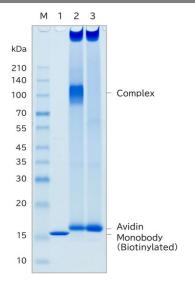


Figure 2. Purity and Biotinylation Efficiency. SDS-PAGE followed by CBB staining was used to analyze the following samples: (1) 1 μ g of the monobody, (2) 1 μ g of the monobody incubated with 5 μ g of avidin, and (3) 5 μ g of avidin alone.

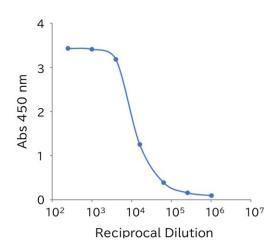


Figure 3. ELISA Reactivity to Ubiquitin Monomer. Serial dilutions of the monobody were assessed by ELISA using plates coated with 1 μ g/well recombinant human ubiquitin monomer. Detection was performed using NeutrAvidin-HRP.



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