

PRODUCT DATASHEET

AVA00100

Anti-diUbiquitin K6 Affimer[®] Reagent – 6xHis tag

Target: di-Ubiquitin (K6-linked)
Avacta clone ID: K6_29

Version: 02
Last revised: May 2018

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC APPLICATIONS.

Applications: WB

Species reactivity: Human

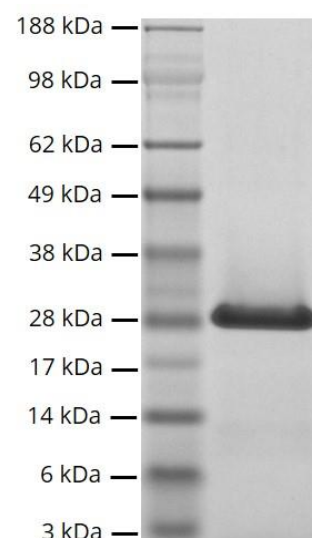
Storage: 4°C

Target Background:

Substrate proteins are linked to ubiquitin via specific lysine residues on ubiquitin itself (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63). Polyubiquitin chains are formed when a lysine residue of one ubiquitin is linked to the carboxy-terminal glycine of another. Poly-ubiquitinated proteins are targeted to a range of different cellular processes; from proteasomal degradation, functional regulation, subcellular localization, to influencing protein-protein interactions. Due to the limited tools available, the exact function of K6-poly-ubiquitination is largely unknown.

Product Information:

Form	Liquid
Storage Instructions	Store at 4°C for up to 6 months. For longer term storage, it is recommended to store at -20°C in single use aliquots.
Buffer	100 mM Sodium Phosphate, 150 mM Sodium Chloride, 0.02% Sodium Azide, pH 7.4
Purity	>95%
Purification method	IMAC followed by desalting
Clonality	Monoclonal
Format	N-terminal 6xHis tag and 3C cleavage site



Source/Purification:

This monoclonal Anti-diUbiquitin K6 Affimer[®] reagent was produced by IPTG-induced expression in E. coli and purified from cell lysates using IMAC chromatography followed by desalting. Purity is >95% by SDS-PAGE (see, right).

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Specificity and Sensitivity:

Anti-diUbiquitin K6 Affimer[®] reagent detects K6 linked poly-ubiquitin chains with a K_D of approximately 20 pM^[1]. Binding to other di-ubiquitin linkages was found to be negligible.

Application Example - Western Blot:

SDS-PAGE & Blotting: Protein samples (200 ng per well) were separated on a Bolt 4-12% Bis-Tris Plus gel (Thermo Fisher) under non-reducing conditions and blotted onto a nitrocellulose membrane using an iBlot2 device (Thermo Fisher).

Blocking: 1 x Casein Block (Sigma) in 1 x TBS, 1 h, RT.

Wash 1: 3 x 5 mL of 1 x TBS-T (0.05% Tween-20), 5 min, RT.

Affimer[®] Incubation: 1 in 1000 in 1 x Casein Block (Sigma) in 1 x TBS, 16 h, 4°C.

Wash 2: 3 x 5 mL of 1 x TBS-T (0.05% Tween-20), 5 min, RT.

Detection: 1 in 1000 Penta-His HRP (BioRad) in 1 x Casein Block (Sigma) in 1 x TBS, 1 h, RT.

Wash 3: 3 x 5 mL of 1 x TBS-T (0.05% Tween-20), 5 min, RT.

Substrate: ECL (Amersham)

Western blots demonstrate the Anti-diUbiquitin K6 Affimer[®] reagent specifically recognises K6 linked di-ubiquitin (~16kDa) over other poly ubiquitin linkages or monomeric ubiquitin.

Key

M: SeeBlue Plus 2 Pre-Stained Standard (Thermo Fisher)

K6: K6-linked di-ubiquitin (Boston Biochem Inc)

K11: K11-linked di-ubiquitin (Boston Biochem Inc)

K29: K29-linked di-ubiquitin (Boston Biochem Inc)

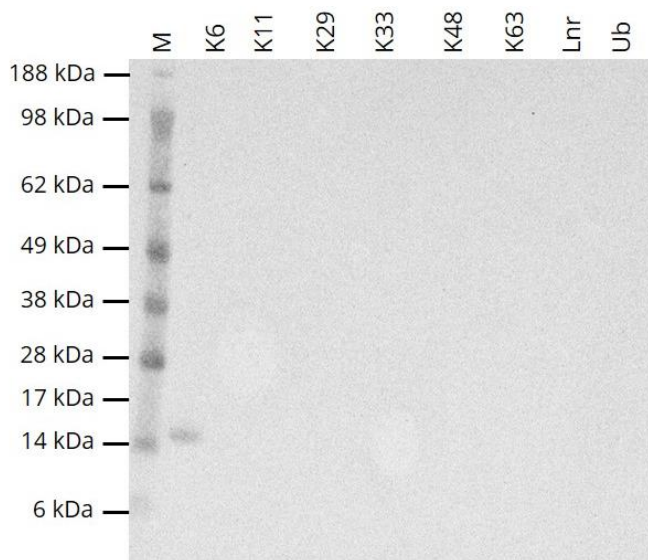
K33: K33-linked di-ubiquitin (Boston Biochem Inc)

K48: K48-linked di-ubiquitin (Boston Biochem Inc)

K63: K63-linked di-ubiquitin (Boston Biochem Inc)

Lnr: Linear di-ubiquitin (Boston Biochem Inc)

Ub: Mono-ubiquitin (R&D Systems)



^[1] Michel *et al*, Ubiquitin Linkage-Specific Affimers Reveal Insights into K6-Linked Ubiquitin Signaling; Molecular Cell 2017 (DOI: <http://dx.doi.org/10.1016/j.molcel.2017.08.020>).