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Product Highlight

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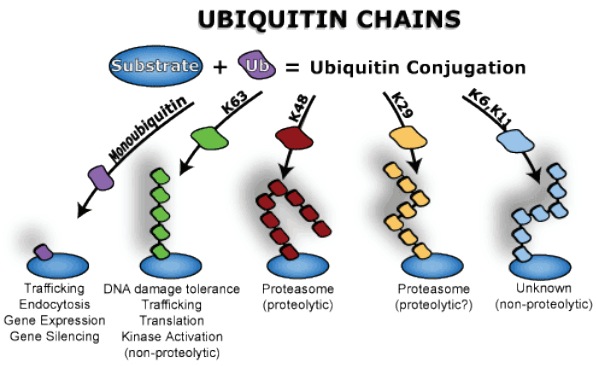
FEATURED CONTENT:

UBIQUITIN CHAINS • SUMO CHAINS • AFFINITY MATRICES (UIM,SIM)

UBIQUITIN CHAINS



The type and number of poly-ubiquitin chains that are conjugated to a target is highly regulated to generate distinct signals that affect different physiological processes. This versatility arises from the fact that not only can targets be mono-ubiquitinated or poly-ubiquitinated, but also that different types of poly-ubiquitin chains are formed. Proteins tagged with ubiquitin are most often destined for degradation by the proteasome. However, mono-ubiquitination and poly-ubiquitination also has non-proteasomal regulatory functions like targeting proteins to nucleus, cytoskeleton and endocytic machinery, or modulating enzymatic activity and protein-protein interactions.

Multi-ubiquitin chains are built by formation of an isopeptide bond between Gly76 of one ubiquitin to the ε-NH2 group of one of the seven potential lysines (K6, K11, K27, K29, K33, K48 or K63) of the preceding ubiquitin. Specific E2 enzymes and E2/E3 combinations result in the formation of linkage-specific ubiquitin chains. Residue K48 is a major site of chain initiation and K48-linkages are highly abundant, being the predominant signal for proteins destined for degradation by the proteasome. The other principle and relatively abundant poly-ubiquitin chain has K63-linkages. K63-linkages do not seem to play a role in protein turnover and have been implicated in receptor endocytosis and sorting, translation, DNA damage repair, the stress response and signaling.

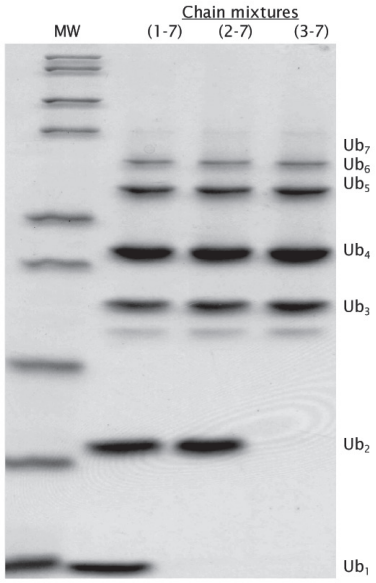


Figure 1. K48-linked ubiquitin chain mixtures (Ub_{1-7}, UC-240), with mono-Ub removed (Ub_{2-7}, UC-230), and with both mono- and di-Ub removed (Ub_{3-7}, UC-220).

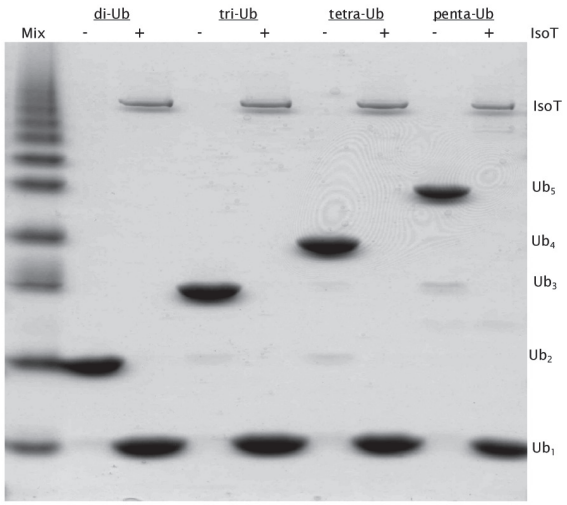


Figure 2. Deconjugation of K63-linked ubiquitin chains by Isopeptidase T to generate mono-ubiquitin. Chain species (4μg) were incubated with enzyme for 30 minutes at 37°C in 50 mM Hepes pH 8.0, 100 mM NaCl, 5mM DTT. (Ub_2,UC-300; Ub_4,UC-310; Ub_3,UC-315; Ub_5, UC-316)

UBIQUITIN CHAINS

- **Linkages**
  Lysine 48 (K48), Lysine 63 (K63) or linear fusion
- **Lengths**
  From mono Ub to high molecular weight chains
- **Bulk Quantities**
- **Custom Ub Chains**
  Using affinity tagged, labeled and/or mutant Ub to form chains with different linkages and lengths

K48-Linked Chains:	Catalog No.	Size
Di-Ub WT Chains (Ub_2)	UC-200	100 µg
Tetra-Ub WT Chains (Ub_4)	UC-210	25 µg
Tetra-Ub WT Chains (Ub-K63-Ub-K48-Ub-K63-Ub)	UCM-210	25 ug
Tri-Ub WT Chains (UB_3)	UC-215	100 µg
Penta-Ub WT Chains (Ub_5)	UC-216	25 µg
Poly-Ub WT Chains (Ub_{3-7})	UC-220	100 µg
Poly-Ub WT Chains (Ub_{2-7})	UC-230	100 µg
His_6-Poly-Ub WT Chains (Ub_{2-7})	UCH-230	100 µg
Poly-Ub WT Chains (Ub_{1-7})	UC-240	100 µg

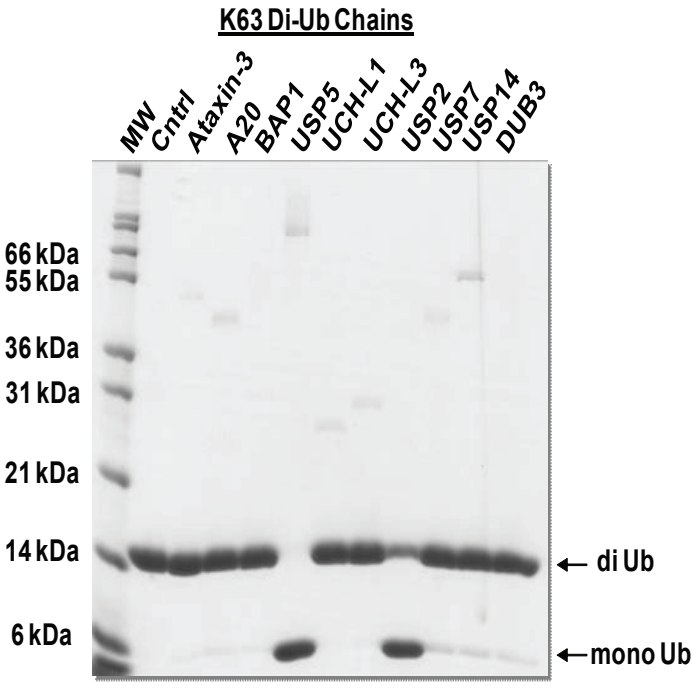


Figure 3. Hydrolysis of K63-linked Ub chains by DUBs. DUBs were incubated with 4µg di-ubiquitin linked through K63 (UC-300).

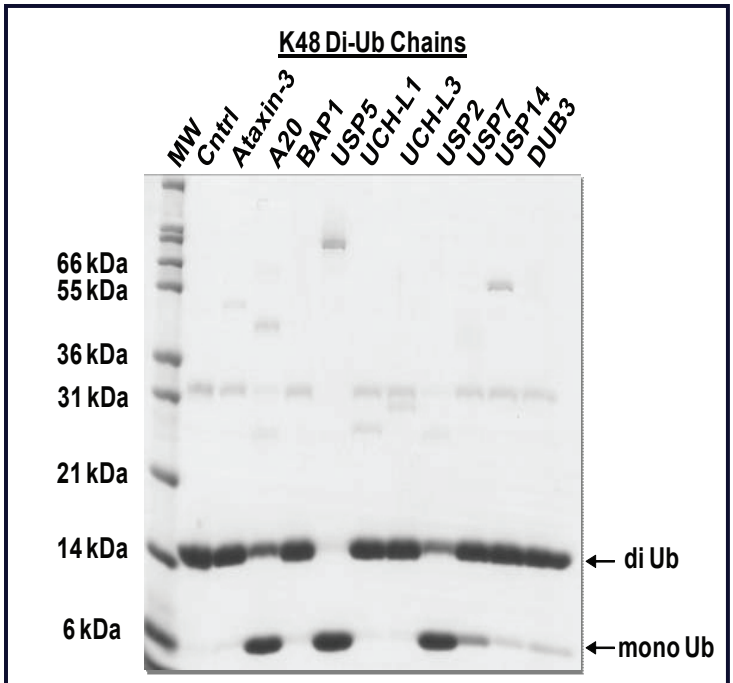


Figure 4. Hydrolysis of K48-linked Ub chains by DUBs. DUBs were incubated with 4µg di-ubiquitin linked through K48 (UC-200).

K63-Linked Chains:	Catalog No.	Size
Di-Ub WT Chains (Ub_2)	UC-300	50 µg
Tetra-Ub WT Chains (Ub_4)	UC-310	25 µg
Tetra-Ub WT Chains (Ub-K48-Ub-K63-Ub-K48-Ub)	UCM-310	25 ug
Tri-Ub WT Chains (UB_3)	UC-315	100 µg
Penta-Ub WT Chains (Ub_5)	UC-316	25 µg
Hexa-Ub WT Chains (Ub_6)	UC-317	25 µg
Octa-Ub WT Chains (Ub_8)	UC-318	25 µg
Poly-Ub WT Chains (Ub_{3-7})	UC-320	100 µg
Poly-Ub WT Chains (Ub_{2-7})	UC-330	100 µg
His_6-Poly-Ub WT Chains (Ub_{2-7})	UCH-330	100 µg
Poly-Ub WT Chains (Ub_{1-7})	UC-340	100 µg
Other Linkages:	**Catalog No.**	**Size**
Linear Di-Ubiquitin (Ub_2)	UC-700	100 µg

Linkage specific poly-Ubiquitin chains are used to investigate mechanisms of binding and recognition by E1 activating or E2 conjugating enzymes, deubiquitinating enzymes, E3 ligases or other proteins that contain ubiquitin interacting motifs (UIMs). Chain products are formed with wild-type ubiquitin and linkage-specific conjugating enzymes.

SUMO CHAINS

- **SUMO-2 and SUMO-3 Chains**
- **Bulk Quantities**
- **Custom Chain Synthesis**

Similar to ubiquitin, the small ubiquitin-related modifier (SUMO) can form polymeric chains on many of its targets, and the poly-SUMO signal is responsible for a number of specific biological functions. Similar to ubiquitination, SUMOylation is a reversible process performed by SUMO-specific proteases (SENPs) which function in both the maturation of SUMO precursor proteins, the disassembly of SUMO chains, and in the removal of SUMO from modified substrates.
We offer a range of polymeric SUMO chains to investigate the substrate specificity of SENP enzymes. These chains can also be used to investigate the mechanism of binding and recognition by SUMO specific E1 or E2 enzymes, E3 ligases or other proteins that contain SUMO binding domains.

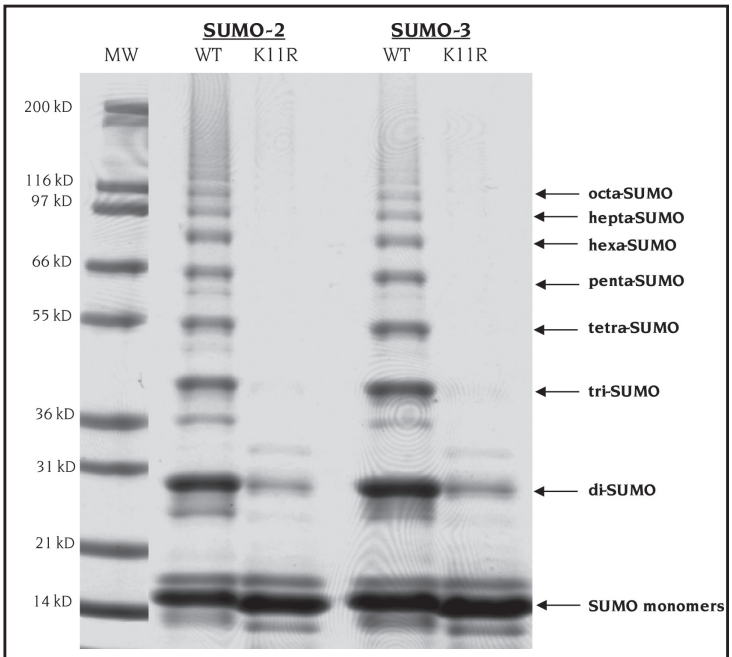


Figure 1. *In vitro* **mediated SUMO-2/3 chain formation is abrogated by the mutation of the conserved lysine 11 to arginine**. SUMO chains were synthesized in the presence of SUMO E1 (E-315) and UbcH9 (E2-645) enzymes. Reaction products were visualized by 16% SDS-PAGE.

SUMO Chains	Catalog No.	Size
di-SUMO-2 WT Chains	ULC-200	50 µg
Poly-SUMO-2 WT Chains (2-8)	ULC-210	25 µg
Poly-SUMO-2 WT Chains (3-8)	ULC-220	25 µg
di-SUMO-3 WT Chains	ULC-300	50 µg
Poly-SUMO-3 WT Chains (2-8)	ULC-310	25 µg
Poly-SUMO-3 WT Chains (3-8)	ULC-320	25 µg

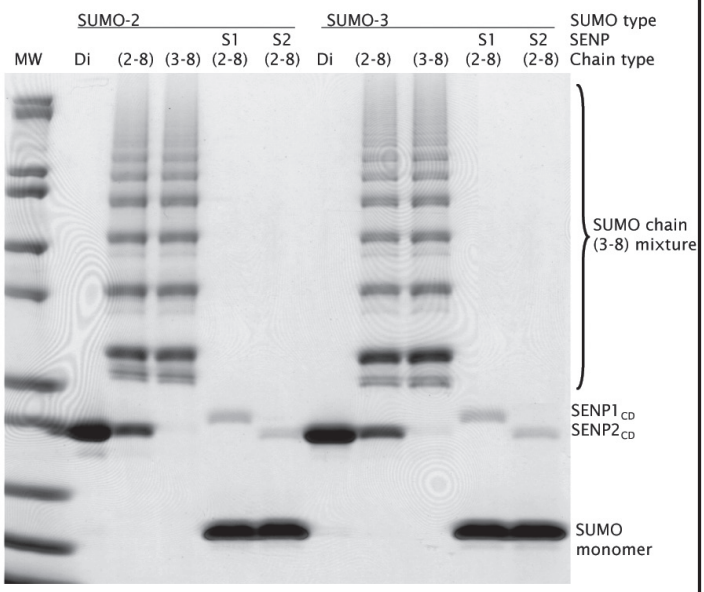


Figure 2. Deconjugation of SUMO-2 and SUMO-3 chains by SUMO-specific isopepidases SENP1 and SENP2. Reactions contained 2µg of SUMO chains (Di-SUMO-2, ULC-200; SUMO-2_{2-8}, ULC-210; SUMO-2_{3-8}, ULC-220; Di-SUMO-3, ULC-300; SUMO-3_{2-8}, ULC 310; SUMO-3_{3-8}, ULC-320) in 50mM Hepes, 50mM NaCl, 5mM DTT and were incubated in the presence of 500nM SENP1 (S1)(E-700) or SENP (S2)(E-710) for 60 minutes at 37°C.

SUMO Mutants	Catalog No.	Size
SUMO-1 K7R	ULM-710	250 µg
SUMO-1 K16R	ULM-712	250 µg
SUMO-1 K17R	ULM-714	250 µg
SUMO-1 K7R, K16R	ULM-716	250 µg
SUMO-1 K7R, K17R	ULM-718	250 µg
SUMO-1 K16R, K17R	ULM-720	250 µg
SUMO-1 K7R, K16R, K17R	ULM-722	250 µg
SUMO-2 K11R	ULM-752	250 µg
SUMO-3 K11R	ULM-762	250 µg

ΨKxE

SUMO-1: ^{1}MSDQEAKPSTEDLGDKKE—GEYIKLKVIGQDS SE IHFKVKMTH LK KLKES^{50}
SUMO-2: ^{1}MAD----EKP------KEGVKTENNDHINLKVAGQDGSVVQFKIKRHTPLSKLMKA^{46}
SUMO-3: ^{1}MSE----EKP------KEGVKTE--NDHINLKVAGQDGSVVQFKIKRHTPLSKLMKA^{45}

SUMO-1: ^{51}YCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTGGHSTV^{101}
SUMO-2: ^{47}YCERQGLSMRQI RFRFDGQPINETDTPAQLEMEDEDTIDVFQQQTGGVY^{95}
SUMO-3: ^{46}YCERQGLSMRQI RFRFDGQPINETDTPAQLEMEDEDTIDVFQQQTGGVPESSLAGHSF^{103}

Figure 3. SUMOylation motif (ΨKxE) is underlined. The precursor forms for each SUMO contains the residues beyond the conserved C-terminal **GG**. Conserved lysines that play a critical role in SUMO polymerization are indicated as (**K**). SUMO-1 contains a total of 11 lysine residues, with some (**K**) not found in SUMO-2 and SUMO-3, which both have a total of 7 lysines. **Primary sequence alignment of SUMO-1, SUMO-2 and SUMO-3 proteins**. Residues in **blue** are identical, to the consensus

AFFINITY MATRICES (UIM, SIM)

Affinity matrices are resins with covalently attached peptides or proteins and are excellent tools for the enrichment, isolation and identification of specific interacting proteins. Boston Biochem offers a variety of matrices which can specifically identify ubiquitin and SUMO chains, and also ubiquitinated or SUMOylated protein substrates. Our reagents have the highest protein:agarose coupling ratios available to maximize binding for low abundance or low affinity proteins of interest.

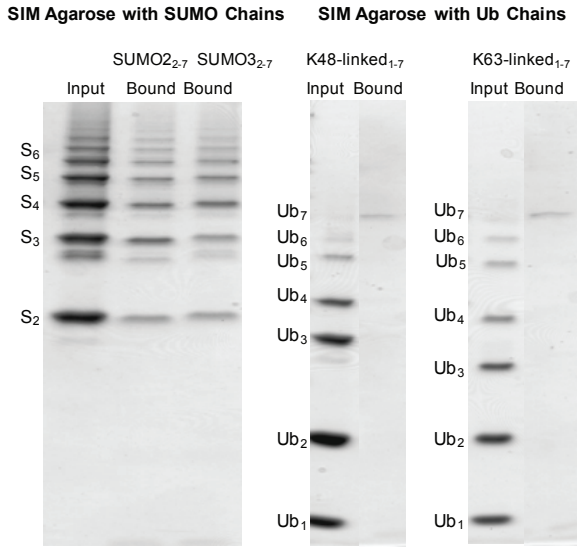


Figure 1. SUMO Interacting Motif (SIM) Agarose binds specifically to SUMO chains. A mixture of either Ub (20µg) (K48 Ub_{1-7}, UC-240; K63 Ub_{1-7}, UC-340) or SUMO(10µg) chains was incubated with 15µl of SIM agarose (AM-200) for 1.5 hours at room temperature. The resin was washed 3 times for 1 minute each with 1ml of wash buffer. The resin was resuspended in 30µl SDS-PAGE sample buffer and heated for 2 minutes at 37°C, and 10µl of supernatant was loaded on a 16% SDS-PAGE gel.

S5a Agarose

AM-100 0.5 ml

S5a (Rpn10) is a component of the regulatory complex (19S) of the 26S proteasome. It binds to and recognizes poly-ubiquitinated proteins and functions as a receptor for proteins destined for proteolytic degradation. The protein recognizes Ub chains and conjugates via two Ub-interacting motif (UIM) domains. S5a has a preference for longer Ub polymers and has a low affinity for mono-, di- and tri-Ub. This affinity resin can be used for the enrichment, isolation and identification of ubiquitinated proteins, 26S substrates or proteins that contain Ub-like domains.

S5a UIM Peptide Agarose

AM-110 0.5 ml

S5a (Rpn10) is a component of the regulatory complex (19S) of the 26S proteasome. It binds to and recognizes poly-ubiquitinated proteins and functions as a receptor for proteins destined for proteolytic degradation. The protein recognizes Ub chains and conjugates via two Ub-interacting motif (UIM) domains located at residues 211-230 (I) and 282-301 (II). Although both UIMs bind to poly-ubiquitin *in vitro*, UIM II has a 10-fold higher affinity for ubiquitin than UIM I. This affinity resin can be used for the enrichment, isolation and identification of ubiquitinated proteins, proteins that contain ubiquitin-like domains and/or 26S substrates.

Ataxin UIM-agarose

AM-115 0.25 ml

Ataxin-3 has deubiquitinating activity *in vitro*. The full-length protein contains a catalytic N-terminal Josephin domain, three ubiquitin interacting motifs (UIMs), and a variable C-terminus with a polyglutamine stretch. Ataxin3 functions as a mixed linkage, chain editing enzyme with preferential cleavage of K63 linkages in mixed chains. Ataxin3 also binds both K48-linked and K63-linked poly-Ub chains via its UIM domains. This affinity resin can be used for the enrichment, isolation and identification of K48-linked or K63-linked poly-Ub chains or ubiquitinated substrates that contain these linkages.

Rap80 UIM-agarose

AM-120 0.25 ml

Rap80 (Receptor Associated Protein 80) interacts with BRCA1, a Ub E3-ligase which functions in conjugation with the BARD1 deubiquitinating enzyme. BRCA1 is recruited to DNA damage sites by poly-ubiquitin chains through Rap80 which contains 2 tandem ubiquitin-interacting motifs (UIMs). The UIM domains of Rap80 have been shown to have preferential binding to K-6 and K63-linked Ub chains. Rap80 has a low affinity for mono-, di- and tri-Ub but binds efficiently to tetra (or greater) Ub chains. This affinity resin can be used for the enrichment, isolation and identification of ubiquitinated proteins that are non-K48-linked.

SUMO-interacting Motif (SIM) Peptide Agarose

AM-200 0.5 ml

Three different amino acid consensus motifs have been identified as mediating SUMO binding and/or interaction. These SUMO-interacting motifs (SIMs) all contain a hydrophobic core sequence with a stretch of acidic amino acids either at the N- or C-terminus. Studies indicate that the SIM hydrophobic region is essential for mediating binding to the α-helix and β2-strand surfaces on SUMO proteins. The negatively charged residues surrounding the hydrophobic core can influence binding affinities and can dictate binding preferences for the various SUMO isoforms. This affinity resin is derived from a PIAS sequence, and can be used for the enrichment, isolation and identification of SUMOylated proteins.

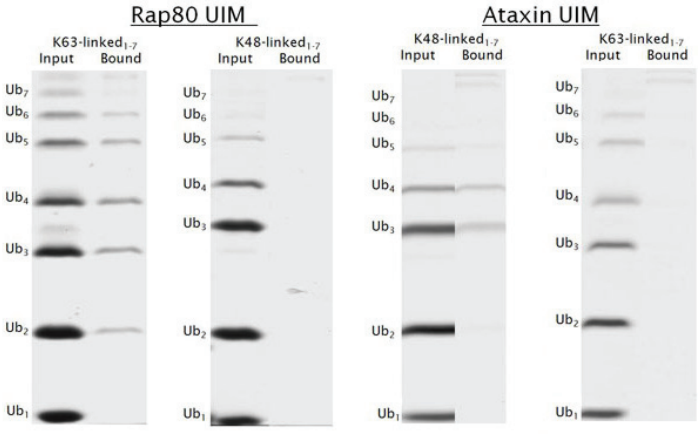


Figure 2. Preferential binding of K63- and K48-linked Ub chains to Rap80 UIM and Ataxin UIM agarose, respectively. A mixture of K63- or K48-linked Ub chains (10µg) was incubated with 25µl Rap80 (AM-120) or Ataxin UIM (AM-115) agarose in a 100µl volume for 1 hour at room temperature. The resin was washed 5 times for 1 minute each with 1ml buffer. The resin was resuspended in 20µl SDS-PAGE sample buffer, 12µl of the supernatant and 2µg of the initial Ub chain input was separated on a reduced 16% SDS-PAGE gel.

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