## K33-linked diUb

## Cat. \# D5410

| Quantity: | $25 \mu \mathrm{~g}$ |
| :---: | :---: |
| Species: | Human |
| Source: | Synthetic |
| MW: | 17107 Da |
| Form: | Lyophilized powder |
| Quality Assurance: | $\geq 95 \%$ by RP-HPLC and SDS-PAGE |
| Sequence: | MQIFVKTLTGKTITLEVEPSDTIENVKAKIODKEGIPPDOORLIFAGKOLEDGRTLSDYNIOKESTLHLVLRLRGG MOIFVKTLTGKTITLEVEPSDTIENVKAKIODKEGIPPDOORLIFAGKOLEDGRTLSDYNIOKESTLHLVLRLRGG |
| Description: | K33-linked diUb is a native K33 linked di-Ub which can be used as a substrate for proteases that cleave the isopeptide linkage between two ubiquitin molecules. It can also be used to investigate mechanism of binding and recognition by proteins that contain ubiquitinassociated domains or ubiquitin-interacting motifs (UIMs). This product is formed by chemical ligation. |

Images


A B C D E F G H I

Coomassie-stained SDS-PAGE of 1 mg each diUb
A: Linear diUb
B: K6-linked diUb
C: K11-linked diUb
D: K27-linked diUb
E: K29-linked diUb
F: K33-linked diUb
G: K48-linked diUb
H: K63-linked diUb
I: Ubiquitin

m/z (amu)


B: LC-MS analysis. Mobile phase $\mathrm{A}=1 \% \mathrm{CH}_{3} \mathrm{CN}, 0.1 \%$ formic acid in water (milliQ) and $\mathrm{B}=1 \%$ water (milliQ) and $0.1 \%$ formic acid in $\mathrm{CH}_{3} \mathrm{CN}$. Phenomenex Kinetex C 18 , $(2.1 \times 50 \mathrm{~mm}, 2.6 \mu \mathrm{M})$; column $\mathrm{T}=40^{\circ} \mathrm{C}$.

Storage:
Sample
Preparation (Important!):

Literature:

Powder at $-20^{\circ} \mathrm{C}$; Solution at $-80^{\circ} \mathrm{C}$. Please avoid multiple freeze/thaw cycles.

1) Centrifuge the tube at $10,000 \mathrm{xg}$ for 2 min to pellet the powder.
2) Dissolve the powder in a small amount of DMSO (e.g. $25 \mu$ g powder in $1 \mu \mathrm{~L}$ DMSO). Vortex the tube to completely dissolve the powder. Keep under room temperature for 5 min , and then centrifuge under room temperature at $10,000 \mathrm{xg}$ for 2 min to collect solution to the tube bottom.
3) Add $49 \mu \mathrm{~L}$ colde buffer (such as 20 mM Tris, pH 7.2, 150 mM NaCl and $10 \%$ glycerol) directly into the tube bottom in once, and pipette up and down to mix (avoid generating bubbles and note the order of addition).
4) The stock solution is $0.5 \mu \mathrm{~g} / \mu \mathrm{L}(29 \mu \mathrm{M})$.
1. A. Faesen et al. , (2011) Chemistry \& Biology, 18, 1550.
2. I. Dikic et al. , (2010) Nature Reviews Molecular Cell Biology 10, 659.
3. J. D. F. Licchesi et al. , (2012) Nature Structural \& Molecular Biology 19, 62.
4. F. El Oualid et al. , (2010) Angewandte Chemie Int. Ed. 49, 10149.
