UbiQ
targeting the ubiquitin system

diubiquitin cleavage assay

version	V1, 17-9-2015
applicable for	UbiQ Chains
reference	Faesen, A.C., et al. The Differential Modulation of USP Activity by Internal Regulatory Domains, Interactors and Eight Ubiquitin Chain Types. *Chem. Biol.* **18,** 1550-1561 (2011).

protocol 1: time-course analysis

1. The reaction mixture contains 50 mM HEPES buffer at pH 7.5 (containing 100 mM NaCl, 1 mM EDTA, 5 mM dithiothreitol, and 0.05% (w/v) Tween-20 with constant enzyme concentration (75 nM)), UAF1 in a 2-fold excess (150 nM), GMPS in a 1:1 stoichiometry and 5μM of di-Ub topoisomer fraction (either linear, K6, K11, K27, K29, K33, K48, and K63). Di-Ub hydrolysis reactions are performed at 37 °C by adding USPs of interest (75 nM).
2. Stop the reactions on different time intervals (0, 5, 10, 30, 60 and 180 min) by adding SDS loading buffer. Analyse the samples from each point on Coomassie-stained SDS-PAGE gels.
3. Analyse the time-course assay by SDS-PAGE analysis.

Protocol 2: kinetic analysis

1. Preheat the reaction mixture 37 °C degrees. The reaction is started by adding a USP of interest.
2. Run the samples on a 12% Bis-Tris NuPage gel (duplicates on one gel), and perform western blots with anti-Ub antibody. The ChemiDoc system (Biorad) can be used to read chemiluminence signal.
3. Mono-Ub can be quantified using the quantification tools of ImageLab (Biorad) using a marker of known amount of mono-Ub and the non-saturated di-Ub signal (including a correction for the amount of di-Ub converted to mono-Ub).

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