Lot No.
KM5685-02-01

ULP1 Technical Data Sheet

Background

A large family of cysteine proteases known as deubiquitinating enzymes (DUB) is capable of dissociating ubiquitin or ubiquitin-like proteins from proteins that have been conjugated to ubiquitin. Ulp1 peptidase cleaves at the Gly-Gly-↓Ala-Thr-Tyr site downstream of the small ubiquitin-like modifier (SUMO) tag.The optimal temperature for cleavage is 30°C; however, the enzyme is active over wide ranges of temperature (see table on page 3) and pH (pH 7.0–9.0). Following digestion, SUMO Protease is easily removed from the cleavage reaction by affinity chromatography using the polyhistidine tag at the N-terminus of the protease. Ulp1 protease is purified from E. coli by affinity chromatography using the polyhistidine tag.

Usage notes:

Item	Amount (µL)
ULP1 Protease	500
SUMO-tagged protein	100
Total volume	600

Table 1: The amounts added for the remaining experiments.

Item	Amount (µL)
ULP1	33
SUMO-tag	13.5
SUMO-tagged protein X	12.2

Table 2: The sizes of items for the remaining experiments.

Storage: ULP1 proteases at –20°C (after first-time use) or at –80°C for long-term storage. Please aliquot to avoid multiple freeze/thaw cycles at –80°C.

Expiration: Stable for 2 years at -80°C from date of shipment.

Guideline for Cleavage

1.Add the ingredients that are shown in below into a microcentrifuge tube:

- ➔ SUMO-Tagged Protein
- ➔ SUMO Protease

2.Mix the sample and incubate it at +4°C in the dialysis buffer.

3. Analyze the sample by SDS-PAGE using a suitable gel.

!! The dialysis buffer should be chosen based on your protein of interest!

!! Load your samples to SDS-PAGE according to the concentration of your protein!

The percentage of protein cleavage is determined by analyzing the amounts of cleaved products (Protein X) and remaining uncleaved protein (SUMO-tagged Protein X) after digestion. Optimizing the percentage of protein cleavage can be achieved by adjusting initial conditions based on the analysis of cleaved and uncleaved protein amounts. To tailor the cleavage process to your specific protein of interest, consider altering the incubation temperature (either +4°C or room temperature), the ratio between enzyme and fusion protein (options include 1:100, 1:500, 1:1000, 1:5000, and 1:10000), and the detection time necessary for the cleavage reaction to occur (ranging from 0.5 to 5.5 hours).

SDS PAGE for Ulp1 cleavage:

Temperature	**Ratios (Protein:Enzyme)**				
+4°C	1:100*	1:500	1:1000	1:5000	1:10000
Room Temperature	1:100*	1:500	1:1000	1:5000	1:10000

Table 3: The ratios used for the remaining experiments.

You can observe the intervals of enzyme digestion time at different temperatures as shown in the 15% SDS-PAGE results in Figures 1 and 2, with a 1:100 ratio.

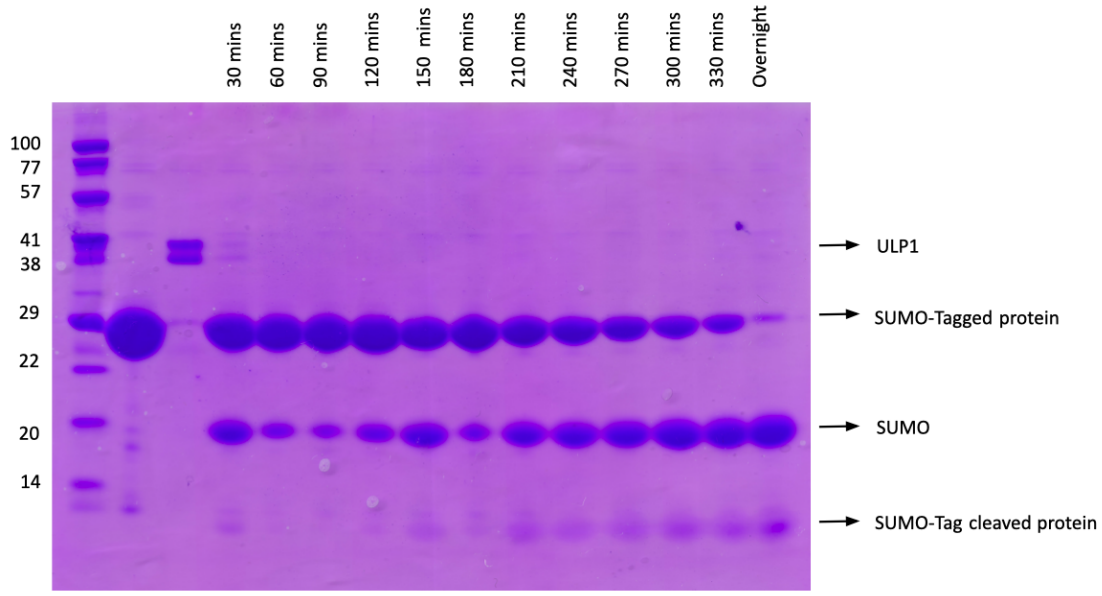

Figure 1: SUMO-Tagged Protein Cut with ULP1 at 4°C

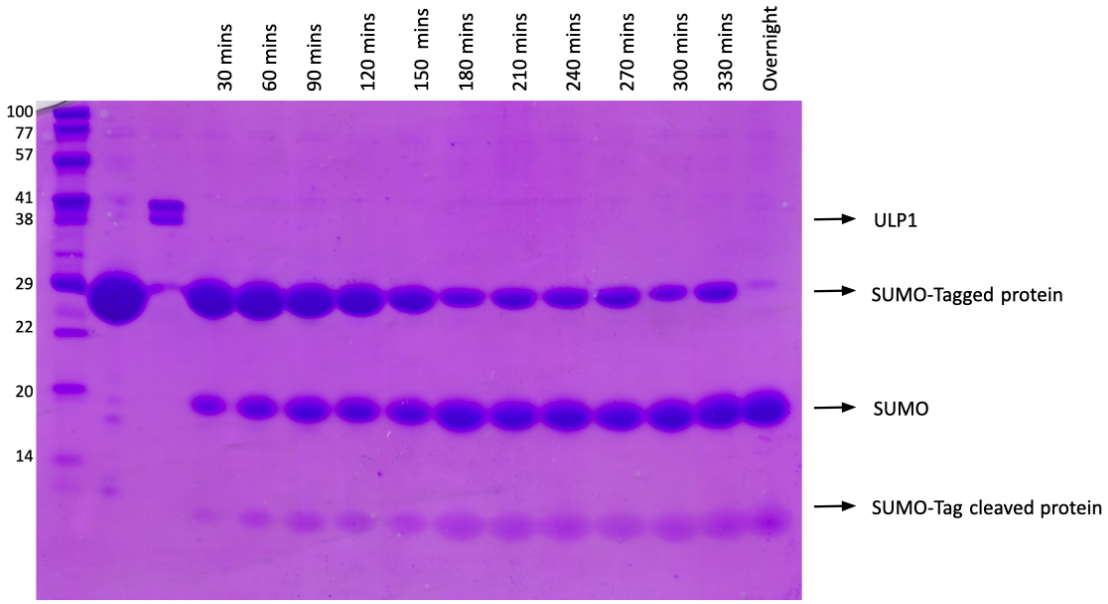

Figure 2: SUMO-Tagged Protein Cut with ULP1 at Room Temperature (RT)

SDS-PAGE Gel Results: The Ulp1 cleavage test was performed on a Sumo-tagged protein (29 kDa) at a 1:100 ratio.

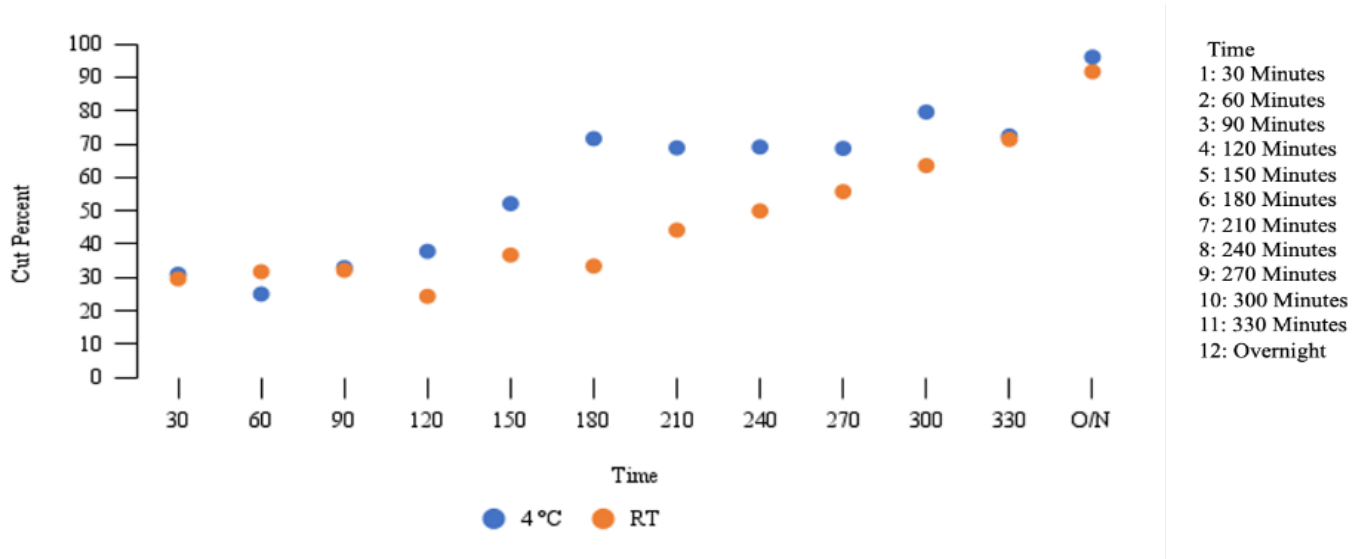

Figure 3: Enzymatic Cut Percent Comparison of 4°C and Room Temperature (RT)