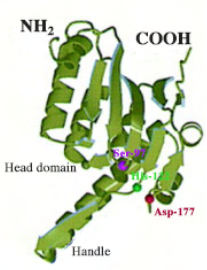
Certificate of Analysis

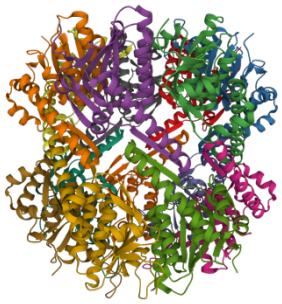
PROTEIN NAME: *E. coli* ATP-dependent Clp protease proteolytic subunit protein (ClpP) His-tag Recombinant (wildtype WT) ClpP expressed in *E. coli*

PROTEIN FACTS	
Number of amino acid (aa) (including 9aa His-tag)	mature monomer protein (202 aa). Proprotein length (216 aa) encompasses prosequence (14 aa).
Purification Tag	His-tag (AAAHHHHHH) at ClpP C-terminus (9aa, 1054.10 Da)
Molecular weight (include His-tag)	22598.95 Da (mature monomer) 316385.3 Da (mature tetradecamer)
Theoretical Isoelectric Point pI	6.12 (mature His-tag monomer) 5.55 (mature non-His-tag monomer)
Extinction coefficient (280nm)	9065 M^{-1} cm^{-1} and Abs 0.1% (=1 g/l) 0.420 (assuming all pairs of Cys residues form cystines) for both mature His-tag monomer and mature non-His-tag monomer
Concentration	397 µg/mL
Storage and Handling	At first use, aliquot and store at -80°C. Avoid multiple freeze-thaws.
Storage Buffer	50mM Tris (pH 8.0), 100 mM NaCl, 10% (v/v) glycerol.
Shipping Conditions	Frozen state on dry ice



Active Monomer
Catalytic triad: Ser-97, His-122 and Asp-177

Tetradecamer (side view)



PROTEIN DESCRIPTION

ClpP is a highly conserved serine protease that forms the proteolytic subunit of the *Escherichia coli* ATP-dependent protease Clp, together with ClpX or ClpA ATPases (Yu & Houry, 2007). Clp protease helps to degrade non-functioning proteins in the cell thereby maintaining protein homeostasis (Yu & Houry, 2007).

Synthesized as a proprotein, ClpP folds into monomer and assemble into two heptameric rings that intercalated back-to-back to form a tetradecameric barrel-shaped peptidase (Wang, Hartling, & Flanagan, 1997). ClpP is activated in-vivo with autocatalytic cleavage of N-terminal regulatory 14-aa prosequence via association with ClpA or ClpX that stack onto one or both ends of ClpP (Maurizi, Singh, Thompson, Kessel, & Ginsburg, 1998; Wang et al., 1997).

Each ClpP monomer housed a catalytic Ser-His-Asp triad (Maurizi, Clark, Kim, & Gottesman, 1990; Wang et al., 1997). Alone, ClpP can slowly degrade very small (less than six amino acids), unfolded peptides (Wang et al., 1997). Processing of larger peptides requires the association of ClpA or ClpX that chaperone, unfold and translocate these peptides into the protease catalytic chamber through the narrow axial pores at both ends of the ClpP barrel and the hydrolysis of ATP (Lee, Baker, & Sauer, 2010). The Clp protease degrades large proteins to 7-8 residues with no apparent sequence specificity (Yu & Houry, 2007).

PROTEIN SEQUENCE: GenBank: CDY55496.1 with added His-tag
mature protein sequence with prosequence propeptide, catalytic triad and His-tag highlighted

MSYSGERDNF APHMALVPMV IEQTSRGERS FDIYSRLLKE RVIFLTGQVE
DHMANLIVAQ MLFLEAENPE KDIYLYINSP GGVITAGMSI YDTMQFIKPD
VSTICMGQAA SMGAFLLTAG AKGKRFCLPN SRVMIHQPLG GYQGQATDIE
IHAREILKVK GRMNELMALH TGQSLEQIER DTERDRFLSA PEAVEYGLVD
SILTHRNAAA HHHHHH

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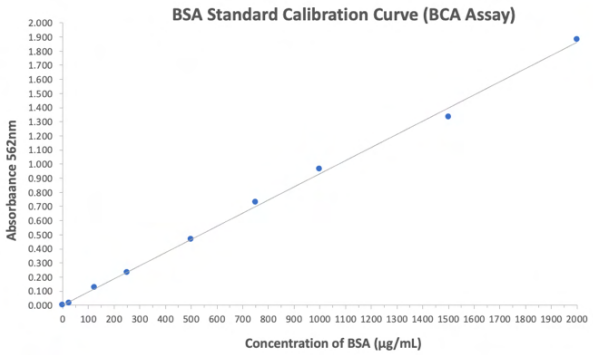
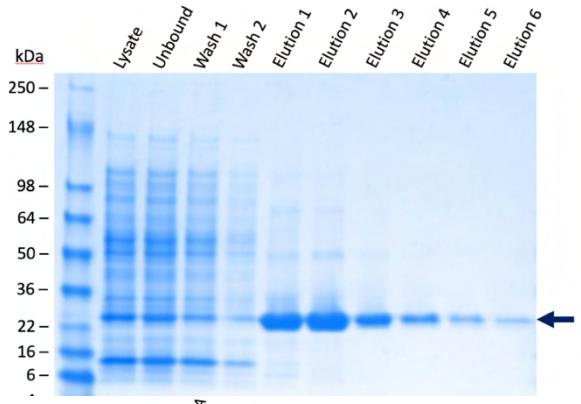
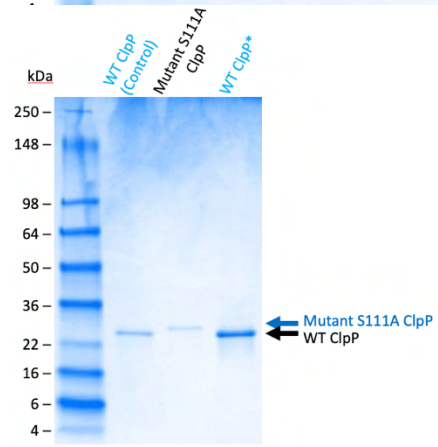
QUALITY CONTROL



Concentration

The concentration of ClpP was determined by Bicinchoninic Acid Assay (BCA) using a standard curve of Bovine Serum Albumin (BSA). The vertical axis represents absorbance at 562nm and the baseline refers to concentration (µg/mL). Average absorbance (0.358) of neat, dilution factor 5 and 10 of ClpP derived concentration of ClpP at 397 µg/mL.

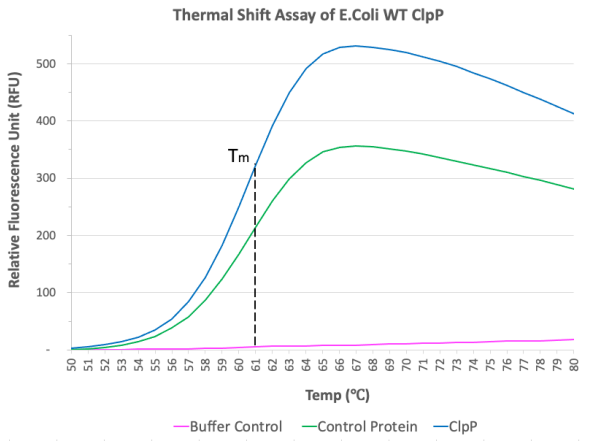




Purity

The purity of ClpP was determined with 4-20% SDS-PAGE gel after purification using immobilized metal affinity purification (IMAC). Lane designations on top of the gel indicate the 10 respective fractions collected progressively, with SeeBlue™ Plus2 Pre-stained Protein Standard molecular weight marker (lane 1). Enriched 22 kDa ClpP was arrow-labelled.

Enriched ClpP protein was further polished using gel filtration to achieve final purity determined with 4-20% SDS-PAGE gel. Lane designations on top of gel indicate SeeBlue™ Plus2 Pre-stained Protein Standard molecular weight marker (lane 1), positive control of another WT Clp (lane 2) and reference mutant 25 kDa S111A ClpP (lane 3, blue arrow-labelled). Enriched 22 kDa ClpP with high purity visible in singular band (WT ClpP* lane 4, black arrow-labelled).



Stability

Protein thermal shift assay using RT-PCR was deployed for stability assessment of ClpP. Melting curves generated against changing temperature (25℃- 99℃) in increments of 1℃ /one-minute cycle for Buffer Control, Control Protein (WT ClpP lab standard) and ClpP. Same buffer (50 mM Tris pH 8.0, 100 mM NaCl, 10% Glycerol) used in all three samples. Melting temperature (Tm) at midpoint of gradient for both ClpP and Control was 61℃, indicating ClpP is stable. Negative Buffer control showed no fluorescence activity.

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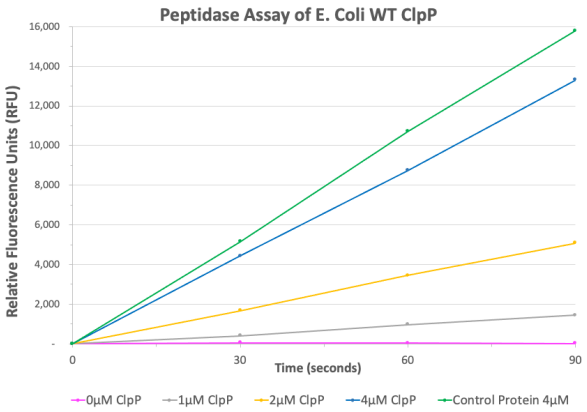

Activity

ClpP was assayed in 100 mM Tris pH 7.5, 600 mM NaCl, 40 mM MgCl2, 20% glycerol and 180mM N-suc-Leu-Tyr-4-AMC peptide substrate. Gradient showed speed of proteolytic activity. Positive Control Protein 4μM (WT ClpP lab standard) and negative control (assay buffer 0μM Clp) showed affirmative and null proteolytic activity respectively. Three ClpP concentration (1μM, 2μM and 4μM) with increasing linear gradients showed active proteolytic activity, which are proportionate to protein concentration.

References

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