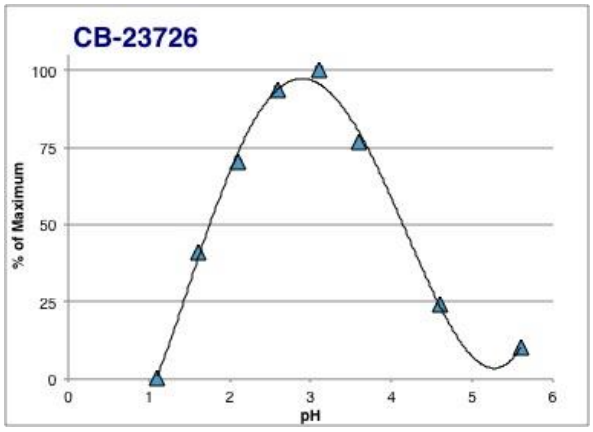
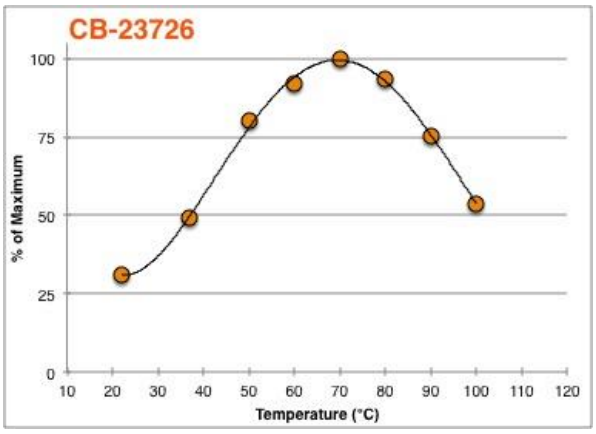
HTA-Enzyme Data Sheet
(For Research Purposes Only)

Product Summary

Product	Class	Optimal pH	pH Range	Optimal Temp.	Temp. Range	Half-life (@ 80°C, pH 3)
Krakatoa CB-23726	HTA-Protease	3.0	*1.8-4.2	70 °C	*40-100 °C	144h (6 days)

* Temperature and pH ranges are conditions that give ≥ 50% maximal activity at optima for other conditions.





HTA-Enzyme storage

DO NOT FREEZE. Hyperthermoacidic Archeal proteases (HTA-Proteases) are prepared and shipped in aqueous 60mM phosphate/citrate buffer at pH=3.0, ready to use, and can be stored for >2 years at room temperature without significant loss of activity. No aliquoting is necessary and low-bind tubes should be used to avoid enzyme adhesion/loss. Reasonable care should be taken to avoid fungal contamination of the enzyme aliquots.

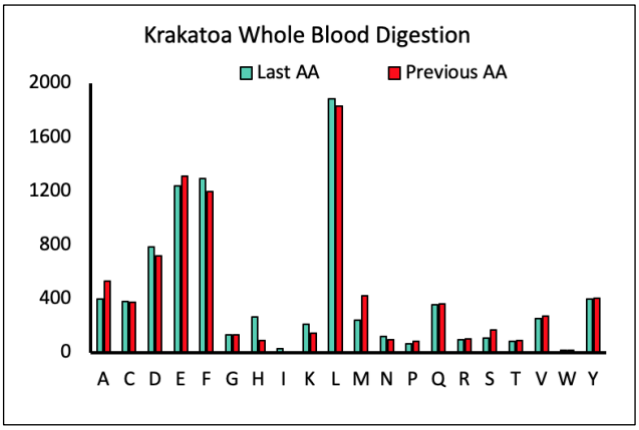
Standard Reaction Conditions

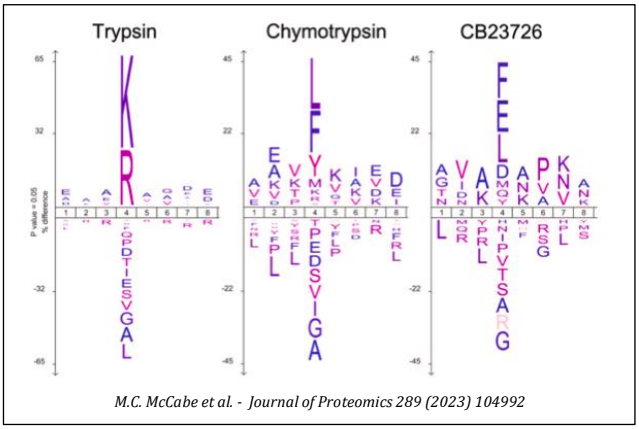
For Proteomics: see our detailed proteomics protocols and published methods at CinderBio.com.

General Proteolysis: HTA-Proteases function optimally at ~80°C and pH=3.0 and effectively digest proteinaceous samples of all kinds. Any acid or derived acidic buffer can be used with HTA-Proteases (Nitric, Phosphoric, Acetic, Peracetic, Hydrochloric, Formic, or Citric acids/buffers) given a pH of 3.0 is achieved and maintained. HTA-proteases show minor or no loss of activity in salts up to 250mM (NaCl, KCl) and tolerate detergents up to ~0.5% (Tween20, NP40, Saponin, Triton x-100, Sarkosyl) with SDS impacting activity most severely with observably diminished activity starting at 0.05%. Phosphate/citrate buffers are weak reductants but 5mM DTT or TCEP does not negatively impact protease activity. Enzyme dosages should be empirically determined for any non-standard reactions or conditions.

Cleavage characteristics - Krakatoa (CB-23726)

Krakatoa (CB-23726) primarily cleaves on the amino-side of glutamic acid (E), leucine (L), and phenylalanine (F) residues with lesser cleavage at other amino acids. HTA-Protease digestions ≥ 2 h can lead to chemical hydrolysis at aspartic acid (D), methionine (M), tyrosine (Y), and glutamine (Q) residues. Krakatoa specificity





is akin to that observed with chymotrypsin and both are less specific than trypsin.

NOTES

CinderBio's HyperThermoacidic Archaeal (HTA) proteases are extraordinarily active and therefore provided in nanogram quantities. Caution should be taken in transferring enzymes between unblocked tubes as enzyme loss can occur due to protein adherence to plastics at these low concentrations. Additionally, samples should be prepared and added to reaction tubes before the enzyme to avoid adherence of enzyme to the tubes. HTA-Proteases store best at ambient conditions, can tolerate refrigeration, and are significantly inactivated by freeze/thaw cycles. **DO NOT FREEZE**.

REFERENCES (1-4)

1. McCabe MC, Gejji V, Barnebey A, Siuzdak G, Hoang LT, Pham T, Larson KY, Saviola AJ, Yannone SM, Hansen KC. **From volcanoes to the bench: Advantages of novel hyperthermoacidic archaeal proteases for proteomics workflows.** J Proteomics. 2023;289:104992. Epub 2023/08/28. doi: 10.1016/j.jprot.2023.104992. PubMed PMID: 37634627.

2. Nam Y, Barnebey A, Kim HK, Yannone SM, Flint S. **Novel hyperthermoacidic archaeal enzymes for removal of thermophilic biofilms from stainless steel.** J Appl Microbiol. 2023;134(6). Epub 2023/05/23. doi: 10.1093/jambio/lxad106. PubMed PMID: 37218716.

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4. Perez Paneda L, Kadava T, Shamorkina TM, Schulte D, Pribil P, Heidelberger S, Narlock-Brand AM, Yannone SM, Snijder J, Heck AJR. **Deep coverage and extended sequence reads obtained with a single archaeal protease expedite de novo protein sequencing by mass spectrometry.** Cell Syst. 2026;17(4):101536. Epub 2026/03/13. doi: 10.1016/j.cels.2026.101536. PubMed PMID: 41819101; PMCID: PMC13083267.