Tryp-N datasheet

Product: Tryp-N, sequencing grade. Supplied frozen in solution at 0.5 µg/µL.

Catalog number: E-01 / K-01

Description: Tryp-N is a novel, thermostable N-terminal peptidase with specificity for Arg/Lys. Peptides generated by Tryp-N exhibit spectral simplification in MS/MS as all strongly basic centers (the amino terminus, Arg and Lys) are located at N-termini of peptides; most ion current is thus concentrated in N-terminal b-ions.

Format: Tryp-N is supplied frozen at 0.5 µg/µL in 50 mM trimethylammonium acetate containing 2 mM $CaCl_2$ and 0.1 mM $ZnCl_2$. At 55 ºC, this buffer has a pH of 7.4. This buffer will not interfere with isobaric labeling using iTRAQ or TMT.

Storage: Tryp-N should be stored frozen at ≤ -20 ºC.

Protease activity: >140 units / mg protease where 1 unit = 1 mg TCA soluble casein peptide liberated per hour in a 3 hr digestion at 65 ºC of 2.5 mg/mL casein with 1:40 wt/wt protease added in a buffer of 25 mM tirmethylammonium acetate, containing 2 mM $CaCl_2$ and 100 µM $ZnCl_2$ adjusted to pH 7.4 at digestion temperature. Commercial trypsin preparations, depending on the preparation and number of freeze/thaw cycles, show activity levels from 94 – 165 units / mg in the same assay with digestion at 37 ºC in 100 mM triethylammonium bicarbonate.

Specificity: >95% cleavage N terminal to K/R as determined by a Tryp-N digestion of *E. coli* lysate at 55 ºC for 3 hrs in TMA acetate, pH 7.4 containing 0.1% Rapigest, 2 mM $CaCl_2$ and 100 µM $ZnCl_2$. A Mascot no enzyme search of Orbitrap data was analyzed by counting both the N-terminal residue of all identified peptides and the residue immediately following identified databases taken from the sequence database.

Enzyme characteristics

Buffers: Tryp-N is active in a wide variety of buffers, with the exception of buffers known to bind metals. Tris, acetate and MS compatible buffers are all particularly suitable. See Figure 1. Suggested buffer to begin is ammonium acetate, trimethylammonium acetate or triethylammonium acetate.

pH: Tryp-N is active in a broad pH range with an optimum around 7 – 10, though substantial activity remains from pH ~5 – 11. See Figure 2. At higher pH, lysines are unprotonated, leading to a preference for cleavage at arginine (see Figure 3). Recommended pH for digestion is 7.4 or from 6 – 8, where the preference for arginine and lysine is equal.

Temperature: The T_{opt} of Tryp-N is 65 ºC (see Figure 4). As discussed in "Recommended use of Tryp-N," thermal precipitation can occur at this temperature, necessitating either the use of lowered temperatures or the inclusion of detergent.

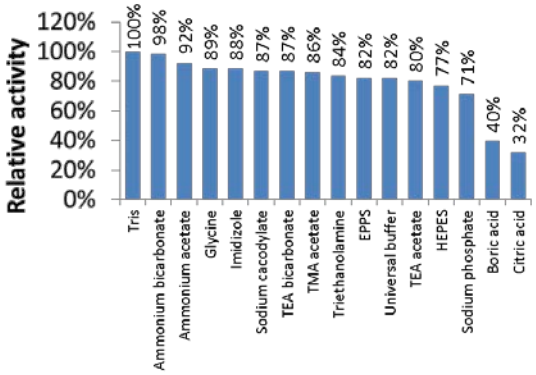

Figure 1 Buffer compatibility. TEA is triethyl-ammonium. TMA is trimethylammonium. All buffers at pH 7.4.

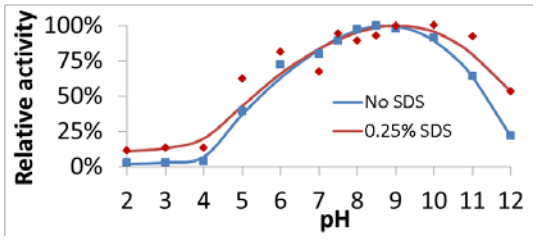

Figure 2 pH_{opt} of Tryp-N. Assay additionally run in the presence of SDS to prevent substrate casein precipitation at low pH.

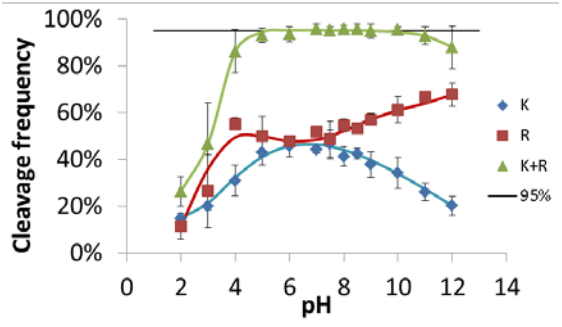

Figure 3 Specificity of cleavage as a function of pH.

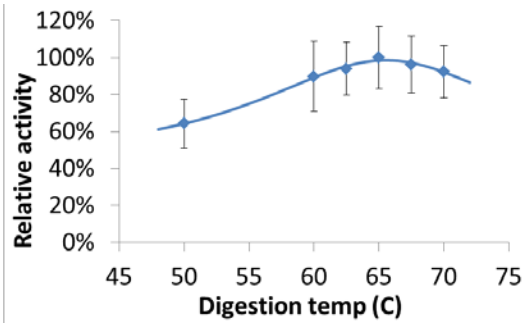

Figure 4 Topt is 65 °C.

Salt: Tryp-N can be inhibited by high ionic strength (Figure 6). Optimization of detergent for particular samples is recommended.

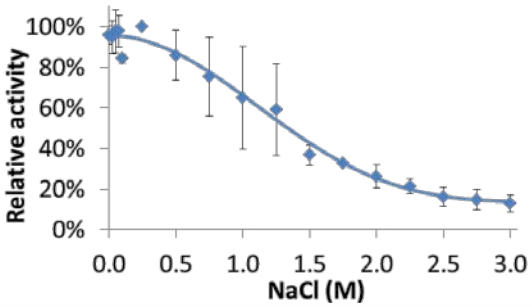

Figure 5 Effect of NaCl on digestion. Run at 50 °C.

Length of digestion: While longer digests are possible, we do not recommend extended incubations at elevated temperatures as peptides may thermally precipitate. At 60 °C, 70-80% of detectable peptides are generated after 30 min. At 60 °C, 0.5 – 4 hr incubation times are optimal. Although cleavage specificity is not significantly decreased, longer incubation times result in fewer identified peptides. See Figure 7.

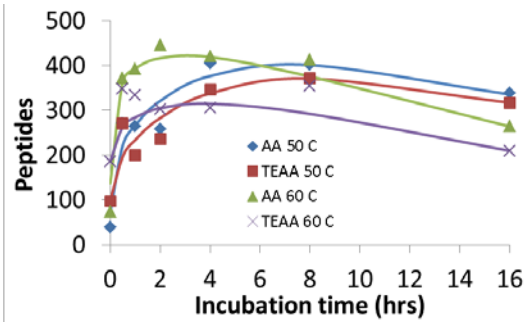

Figure 6 Number of peptides detected as a function of temperature and incubation time.