

## TRYPSIN, MODIFIED *SequENZ*<sup>™</sup>

### *Proteomics/Sequencing Grade*

Trypsin is a serine endopeptidase which specifically cleaves peptide bonds on the carboxy side of arginine, lysine and s-aminoethyl cysteine residues. Poor or no cleavage at arginyl-proline or lysyl-proline bonds is usual. Worthington *SequENZ*<sup>™</sup> modified sequencing grade trypsin is prepared from bovine pancreas and is subjected to extensive purification to remove contaminating non-trypsin proteases and tryptic autolysis by-products which could affect the specificity of the digestion process. Subsequently the enzyme is chemically modified to minimize the autolysis process as well as increase stability. The specificity of the enzyme is routinely checked after chemical modification.

<b>Description</b>	<b>Activity</b>	<b>Code</b>	<b>Cat #</b>	<b>Size</b>	<b>Price</b>
<b><i>SequENZ</i><sup>™</sup> Modified, Sequencing Grade</b> Worthington TPCK-treated trypsin, code TRTPCK, chemically modified to reduce autolysis and increase stability while retaining its specificity. Supplied as a lyophilized powder. Store at 2-8°C.	≥ 4 Casein units per mg protein	TRSEQZ	LS02120 LS02122	4 x 25 ug 4 x 100 ug	\$ 53.00 180.00

### Application Information

Packaging:	25µg and 100µg lyophilized vials.
Reconstitution:	Recommend reconstitution in 1mM HCl, usually at a concentration of 1 µg/µl. Note: Other buffers and concentrations are possible depending on the application.
Working Concentration:	Typically at a ratio of 1/100 to 1/20, the ratio is enzyme to protein, by weight. The calculated amount is added to the protein to be digested.
Stability:	The reconstituted trypsin is stable in 1mM HCl at 2-8°C for up to 6 months. Note: It is recommended that the reconstituted product should be aliquoted and stored at -20°C for longer term storage.
Digestion Buffer:	A standard digestion buffer is 50mM Tris-HCl, 11.5mM CaCl <sub>2</sub> , pH 8.1
Digestion Temperature:	25° - 37°C.
Digestion Time:	Typically 1 hr to 10 hrs, but can extend to 24 hours for some applications. Note: <i>SequENZ</i> <sup>™</sup> modified trypsin is stable for at least 24 hours at 37°C and the auto-digestion products are minimal. It is always preferable to use the shortest incubation time possible, since cleavage, particularly on the carboxy side of hydrophobic residues, has been found to occur at prolonged incubations (more than 8 hours).

(Over) [1.07]

## Stability

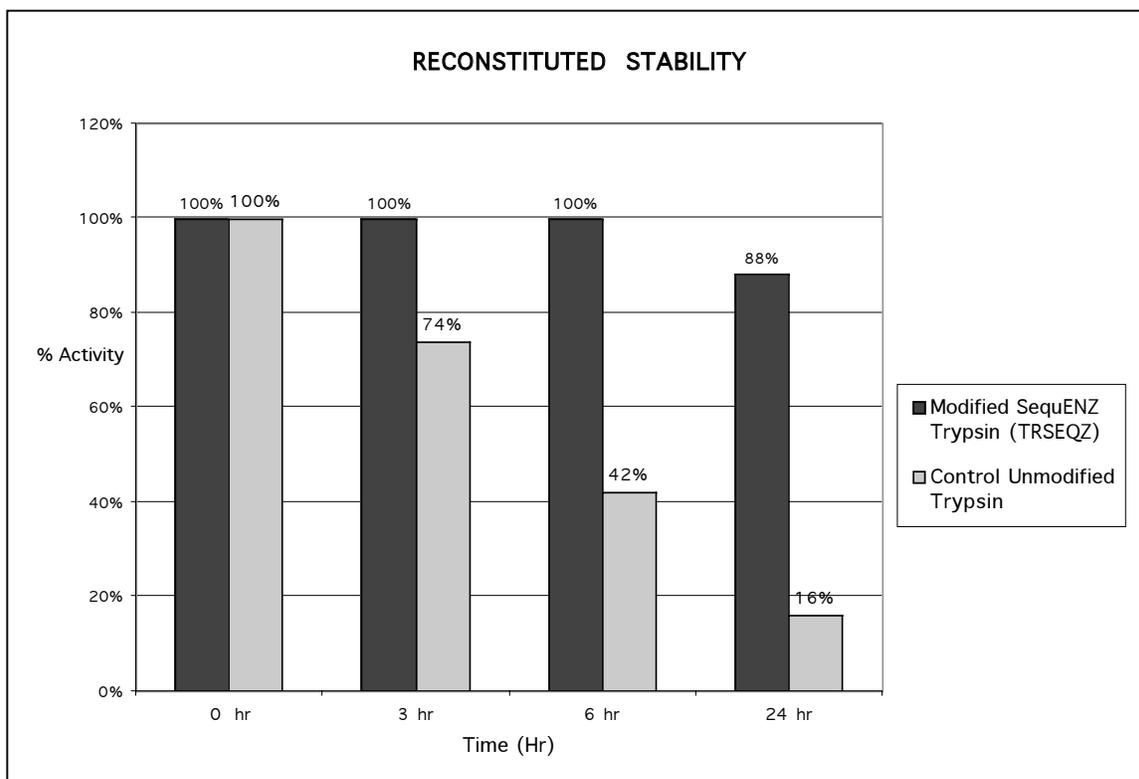
In cases of proteins which are hard to solubilize, denaturing agents such as urea or guanidine-HCl may be added to the digestion buffer prior to the solubilization of the protein. The following table contains data on the effect of these agents on the enzymatic activities of *SequENZ*<sup>TM</sup> modified trypsin at various concentrations:

<u>Denaturing Agent</u>	<u>Concentration</u>	<u>% Enzyme Activity Retained</u>
Control	-	100%
Urea	0.1M	100%
Urea	0.5M	100%
Urea	1.0M	100%
Urea	2.0M	100%
Guanidine-HCl	0.05M	90%
Guanidine-HCl	0.1M	70%
Guanidine-HCl	0.25M	70%
Guanidine-HCl	0.5M	16%

**Conditions:** Measurements were obtained from incubation of 5 $\mu$ g of *SequENZ*<sup>TM</sup> modified trypsin (TRSEQZ) with 1ml of 2% casein in 50mM Tris-HCl, 2mM CaCl<sub>2</sub>, pH 7.8, at 37°C for 1 hour. Activity is measured as  $\mu$ moles tyrosine released in acid-soluble supernatant in the presence of denaturing agents as indicated.

## Effect of Incubation Times, at 37°C, on *SequENZ*<sup>TM</sup> Trypsin Activity

The proteolytic activity of *SequENZ*<sup>TM</sup> modified trypsin was measured after incubation in 50mM Tris-HCl, 11.5mM CaCl<sub>2</sub>, pH 8.1 at 37°C for the specified periods of time. The results are presented in the following chart:



**Conditions:** After the specified incubation times, *SequENZ*<sup>TM</sup> modified trypsin was added to 1ml of 2% casein in 50mM Tris-HCl, 2mM CaCl<sub>2</sub>, pH 7.8. Activity is measured as  $\mu$ moles tyrosine released in the acid-soluble supernatant after digestion for 1 hour at 37°C. Results shown are the average of 5 separate determinations. Note: Unmodified trypsin continues to lose substantial activity after 3 hours incubation at 37°C, under the assay conditions.