SG-Chymotrypsin™

(Cat. # 786-13)

INTRODUCTION

SG-Chymotrypsin™ is a sequencing grade serine endopeptidase, which predominantly cleaves peptide bonds on the carboxy side of tyrosine, phenylalanine and tryptophan. In addition, it has a low catalytic activity against the carboxy side of leucine, methionine, alanine, aspartic and glutamic acids, although at a much lower rate. It is therefore recommended to always use the shortest digestion time possible. SG-Chymotrypsin™ is first treated with TLCK and then subjected to an extensive purification process to remove contaminating protease and chymotryptic autolysis by-products. The highly purified enzyme is then chemically modified to increase its resistance to autolysis and increase its stability. The modified enzyme retains >80% of its activity after 6 hours incubation at 30°C in reaction buffer and >70% of activity after 24 hours incubation under the same conditions. The chemically modified SG-Chymotrypsin™ is stable in denaturing agents (see Table) and therefore can be used to digest difficult to solubilize proteins.

Denaturing Agent	Concentration	% Enzyme Activity Retained
Control	-	100
Urea	0.50M	100
	1.00M	100
	2.00M	100
	3.00M	100
	4.00M	100
Guanidine-HCl	0.05M	100
	0.10M	100
	0.25M	100
	0.50M	11

ITEM(S) SUPPLIED (CAT. # 786-13)

Description	Size
SG-Chymotrypsin™	2 vials, 5µg/vial
Digestion Buffer (CHY)	2ml

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store at -20°C and is stable for 1 year.

PREPARATION BEFORE USE

NOTE: *SG-*Chymotrypsin™ *is supplied lyophilized, 5µg/vial.*

Reconstitute the enzyme with 100µl sterile water to produce a concentration of 100ng/µl. Combine 5µl of 100ng/µl solution with 95µl Digestion Buffer (CHY) to yield 5ng/µl chymotrypsin. Reconstituted enzyme is stable for 1 month at -20°C, repeated freeze thawing is not recommended.

NOTE: *If reduction and alkylation are needed 250mM dithiothreitol and 625mM iodoacetamide are recommended.*

PROTOCOL

For optimal digestion make sure protein sample is either prepared or equilibrated in 50mM Tris-HCl, pH8.0, 0.1mM $CaCl_2$.

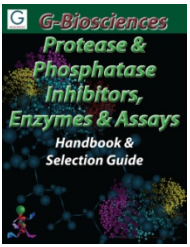
1. For protein fragmentation, 5ng/µl SG-Chymotrypsin™ is typically added to the protein at a ratio of 1:20 to 1:100 enzyme to protein, by weight.

2. The incubation is allowed to proceed at 37°C for 2-10 hours, but can be extended to 24 hours in some applications.

   NOTE: *It is recommended to choose a ratio of enzyme to protein to allow for the shortest incubation time possible, to reduce or eliminate the catalyzed hydrolysis of peptide bonds with non-aromatic amino acid residues.*

RELATED PRODUCTS

Download our Protease & Phosphatase Inhibitors, Enzyme & Assays Handbook.



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