Promega

Proteases for Mass Spectrometry Sample Preparation

Proteases for Mass Spec Sample Preparation

Trypsin and Alternative Proteases for Enhanced Sequence Coverage.

Trypsin

Cleavage with sequence-specific endoproteases is one approach used to identify proteins by mass spectrometry. Following digestion, the generated peptides are analyzed by determination of molecular mass or specific sequence. Trypsin is the favored enzyme for this application because: a) the peptides contain a basic residue (Arg or Lys) on the C terminus and thus are good candidates for collision induced activation (CAD) in tandem experiments (low charge states and high mass-to-charge ratios); b) it is relatively inexpensive; and c) optimal digestion conditions are well characterized. Table 1 illustrates the characteristics and typical reaction conditions for the trypsin protease formulations available from Promega.

Table 1. Characteristics and Reaction Conditions for Trypsin Proteases.

	Trypsin Gold; Sequencing Grade Modified Trypsin; Sequencing Grade Modified Trypsin, Frozen	Immobilized Trypsin	Trypsin/Lys-C Mix
Source and Size	Porcine Pancreas (23.8kDa)	Porcine Pancreas (23.8kDa)	Mix of Tryspin Gold & rLys-C
Cleavage Sites	C-terminal of Lys, Arg. Does not cleave if Lys and Arg are followed by Pro. Asp and Glu at C-terminal side of Lys and Arg inhibit cleavage.	C-terminal of Lys, Arg. Does not cleave if Lys and Arg are followed by Pro. Asp and Glu at C-terminal side of Lys and Arg inhibit cleavage.	C-terminal of Lys, Arg. Does not cleave if Lys and Arg are followed by Pro. In contrast to trypsin, Trypsin/Lys-C efficiently tolerates presence of Glu and Asp at C-terminal side of Lys and Arg.
Protease Protein Ratio (w/w)	1:20 to 1:100	see Technical Manual TM077	1:25 to 1:50
pH Range for Digestion	pH 7–9	pH 5–9	pH 8
Reaction Conditions	50–100mM Tris-HCl (pH 8) or 50–100mM NH_4HCO_3(pH 7.8). Digest overnight at 37°C.	50mM NH_4HCO_3 (pH 7.8). Digest for 30 minutes at room temperature.	50–100mM Tris-HCl (pH 8) or 50mM NH_4HCO_3 (pH7.8). Digest overnight at 37°C.
Buffer Compatibility	Tris-HCl, NH_4HCO_3	Tris-HCl, NH_4HCO_3	Tris-HCl, NH_4HCO_3
In-Gel Digestion Compatibility	Yes	No	Yes
ProteaseMAX™ Compatibility	Yes	Yes	Not tested
Notes	Most widely used protease in mass spectrometry. All Promega trypsin is treated with TPCK to inactivate chymotrypsin contamination and chemically modified (methylated) to minimize autoproteolysis. All Promega trypsin is resistant to mild denaturing conditions (1–2M urea and 0.1% SDS). They retain 48% activity in 2M guanidine chloride.	Used if rapid digestion is required. Urea ≤ 4M; guanidine HCl ≤ 3M; methanol < 60%; DTT ≤ 50mM; TCEP ≤ 5mM; pH 5–9; Iodoacetamide ≤ 300mM.	Trypsin/Lys-C Mix is the most robust trypsin preparation, designed to improve general protein digestion. Trypsin/Lys-C Mix uses the same regular overnight digestion at 37°C under non- or mildly denaturing conditions as trypsin alone.

Alternative Proteases

Increasingly, alternative proteases are being used in combination with trypsin or instead of trypsin in mass spectrometry applications. Use of alternative proteases can increase overall sequence coverage and help locate post-translational modifications. We offer a range of alternatives to trypsin for mass spectrometry analysis. Characteristics and reaction conditions for these proteases are shown in Table 2.

Table 2. Characteristics and Reaction Conditions for Specific Alternative Proteases.

	rLys-C	Endoproteinase Lys-C	Arg-C	Asp-N	Glu-C
Source and Size	*Pseudomonas aeruginosa.* Expressed in *E.coli* (27.7kDa)	*Lysobacter enzymogenes* (30kDa)	*Clostridium histolyticum* (Subunits: 45kDa and 12kDa)	*Pseudomonas fragi* (24.5kDa)	*Staphylococcus aureus V8* (27kDa)
Cleavage Sites	C-terminal of Lys. Does not cleave if Lys is followed by Pro. Asp and Glu at C-terminal side of Lys inhibit cleavage.	C-terminal of Lys. Does not cleave if Lys is followed by Pro. Asp and Glu at C-terminal side of Lys inhibit cleavage.	C-terminal of Arg. Also cleaves at Lys albeit at lower efficiency.	N-terminal of Asp.	C-terminal of Glu. Low level cleavages might occur at Asp residues too albeit at 100–300 fold lower efficiency.
Protease Protein Ratio (w/w)	1:20 to 1:50	1:20 to 1:100	1:20 to 1:350	1:20 to 1:200	1:20 to 1:200
pH Range for Digestion	pH 8–9	pH 7–9	pH 7.6–7.9	pH 4–9	pH 4–9
Reaction Conditions	50-100mM Tris-HCl (pH 8) or 50mM NH_4HCO_3 (pH 7.8). Digest 2–18 hours at 37°C.	50-100mM Tris-HCl (pH 8) or 50mM NH_4HCO_3 (pH 7.8) Digest 2–18 hours at 37°C.	50mM Tris-HCl (pH 7.6–7.9), 5mM $CaCl_2$, 2mM EDTA, >2mM DTT. Digest 2–18 hours at 37°C.	50mM Tris-HCl (pH 8). Digest 2–18 hours at 37°C.	100mM NH_4HCO_3 (pH 7.8), 50–100 mM HCl (pH8). Digest 2–18 hours at 37°C.
Buffer Compatibility	Tris-HCl, NH_4HCO_3	Tris-HCl, NH_4HCO_3	Tris-HCl, NH_4HCO_3	Tris-HCl, NH_4HCO_3	NH_4HCO_3, Ammonium acetate
In-Gel Digestion Compatibility	Yes	Yes	Yes	Yes	Yes
ProteaseMAX™ Compatibility	Yes	Yes	Yes	Yes	Yes
Notes	Inexpensive alternative to a native Lys-C protease. Similarly to a native protease, rLys-C tolerates high denaturing conditions such as 8M urea. Used to digest tightly folded hard-to-digest proteins. Also used as a trypsin alternative if larger peptides are preferable for the analysis. If urea is used in protein sample preparation, avoid high temperature. High temperature induces protein carbamylation in the presence of urea.	Tolerates high denaturing conditions such as 8M urea. Used to digest tightly folded hard-to-digest proteins. Also used as a trypsin alternative if larger peptides are preferable for analysis. If urea is used in protein sample preparation, avoid high temperature. High temperature induces protein carbamylation in the presence of urea.	Used in analysis of histone modifications. Requires DTT, cysteine or other reducing agent and $CaCl_2$ for activity.	Can be used as a trypsin alternative to achieve better cleavage site distribution. Full activity retained in the presence of urea (up to 3.5 M), guanidine HCl (1M), SDS (up to 0.028%), ProteaseMAX™ Surfactant (up to 0.026%), acetonitrile (up to 60%), EDTA (up to 2mM); DTT or ß-mercaptoethanol (up to 5mM).	Can be used as a trypsin alternative to achieve better cleavage site distribution. Glu-C activity and cleavage specificity is affected by buffer conditions. In ammonium biocarbonate and other non-phosphate buffers, Glu-C cleaves C-terminal of Glu. Glu-C cleaves C-terminal of Glu and Asp in phosphate buffer.

Ordering Information

Product	Size	Cat.#
Sequencing Grade Modified Trypsin	100µg (5 × 20µg)	V5111
	100µg	V5117
Trypsin Gold Mass Spec Grade	100µg	V5280
Sequencing Grade Modified Trypsin, Frozen	100µg (5 × 20µg)	V5113
Immobilized Trypsin	2ml	V9012
	4ml (2 × 2ml)	V9013
Trypsin/Lys-C Mix, Mass Spec Grade	20µg	V5071
	100µg	V5072
	100µg (5 × 20µg)	V5073
Arg-C, Sequencing Grade	10µg	V1881
Asp-N, Sequencing Grade	2µg	V1621
Glu-C, Sequencing Grade	50µg (5 × 10µg)	V1651
Endoproteinse Lys-C, Sequencing Grade	5µg	V1071
rLys-C, Mass Spec Grade	15µg	V1671

For more information, visit: **www.promega.com/mass-spectrometry**

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