

Sequencing Grade Proteases

Selective digestion for predictable results



Proteases used for peptide mapping and for characterization of structure domains in proteins, have to be of well defined cleavage specificity and free of contaminating activity such as unspecific proteases. Because of the high specificity, purity and lot-to-lot consistency the Sequencing Grade Proteases supplied by Roche Applied Science are excellent tools for these applications.

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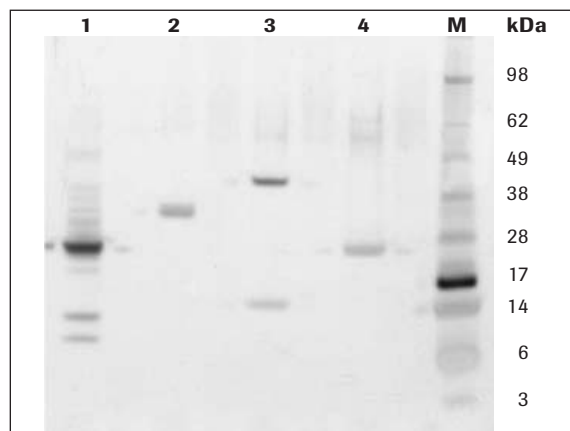


Figure 1: Purity of Roche Applied Science Proteases, Sequencing Grade. SDS-PAGE analysis of endoproteinases followed by silver staining. 100 ng of each endoproteinase were applied on the gel.

Lane M: marker (SeeBlue, Invitrogen)
 Lane 1: Trypsin, Proteomics Grade (23,5 kDa)
 Lane 2: Endoproteinase Sequencing Grade Lys-C (33 kDa)
 Lane 3: Endoproteinase Sequencing Grade Arg-C (59 kDa)
 Lane 4: Endoproteinase Sequencing Grade Asp-N (27 kDa)
 Minimal autolysis was observed

Cleavage Sites

Sequencing Grade Proteases	Cleavage sites	
Carboxypeptidase Y	-Y- ↑ -X-COOH	X,Y = non-specific cleavage reduced if Y = Gly, Asp or X = His, Arg, Lys cleavage enhanced if X or Y = aromatic
Chymotrypsin	-X- ↑ -Y-	Y = non-specific X = Trp, Tyr, Phe cleavage reduced if X = Leu, Met, Ala, Asp, Glu
Endoproteinase Arg-C	-Arg- ↑ -Y-	Y = non-specific
Endoproteinase Asp-N	- ↑ -Asp-Y- and - ↑ -CysSO₃H-Y-	Y = non-specific
Endoproteinase Glu-C	-Glu- ↑ -Y- and -Asp- ↑ -Y-	Y = non-specific
Endoproteinase Lys-C	-Lys- ↑ -Y-	Y = non-specific
Trypsin, Proteomics Grade	-Lys- ↑ -Y- and -Arg- ↑ -Y-	Y = non-specific

Benefit

from our broad experience in enzyme development and optimization, facilitating the success of your experiment and providing optimal results.

Proteases are qualified for mass spectrometry analysis

- Reproducible cleavage pattern of interesting protein is achieved
- Clear mass spectra facilitate sequence confirmation or de novo characterisation

Obtain high specificity

- Cleavage sites are tested with peptides of known sequences, at high protease concentrations (1:10 ratio) and overnight incubations (16 h)

Rely on Purity

- Free of impurities in the separation range of peptides
- Unspecific cleavages are not observed, only expected peptides are present

Benefit from lot-to-lot consistency

- No contamination in peptide sequencing is detected as each lot is purity and specificity tested

Ensure high stability

- Enzymes are supplied with a guaranteed stability for the stated shelf life
- Enzymes are dispatched in aliquots along with precise incubation conditions

Obtain good value

- Pack sizes are divided in aliquots, ensuring that only the quantity needed for each experiment is dissolved

Reduce preparation time

- Preparation of reconstitution buffer is not required
- Lyophilizates are completely dissolved in water, or in the supplied buffer

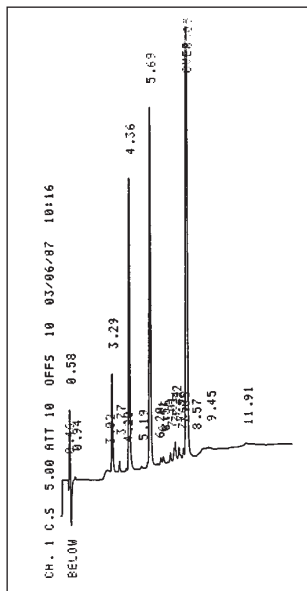


Figure 2: Specificity of Endoproteinase Asp-N Sequencing Grade in reversed phase HPLC.

Digest: 100 µg glucagon + 10 µg Endoproteinase Asp-N Sequencing Grade in 100 µl 50 mM sodium phosphate; buffer, pH 8.0; 18 h at 37°C; reversed phase HPLC: 20 µl digest;

column: Shandon ODS Hypersil, 5 µ; solvent A: 0.1% TFA, (v/v) in water; solvent B: 0.1% TFA, (v/v) in water; 70% acetonitrile, (v/v); gradient: 20 min linearly 0–100% B; flow rate: 1 ml/min; wave length: 215 nm.

Fragments:

3.29 min Asp(15) – Gln(20), 5.69 min Asp(9) – Leu(14),
4.36 min His(1) – Ser(8), 8.05 min Asp(21) – Thr(29).

Denaturing agent	Concentration	Enzyme activity in %
Without addition (control)	-	100
Sodium dodecyl sulfate (SDS)	0.001% (w/v)	113
	0.01% (w/v)	122
	0.1% (w/v)	10
Urea	0.1 mol/l	100
	0.5 mol/l	108
	1.0 mol/l	105
Guanidinium hydrochloride	0.1 mol/l	100
	0.5 mol/l	85
	1.0 mol/l	80
Acetonitrile	1% (v/v)	90
	5% (v/v)	115
	10% (v/v)	125

Table 1: Stability in denaturing agents

Incubation of Endoproteinase Asp-N Sequencing Grade 200 µg/ml, with denaturing agents for 6 h at 25° C in sodium phosphate buffer, 25 mmol/l, pH 7.8. Activity determination of Endoproteinase Asp-N Sequencing Grade with azocoll as substrate.

Product Overview and Description

Benefit from our high-quality enzymes for optimal results

Protease Origin / EC. No. / Molecular weight	Specificity	Reconstitution	Suggested working concentration
Carboxypeptidase Y Sequencing Grade from yeast EC 3.4.16.1 M _r = 61 kD	Serine carboxypeptidase - Y - ↑ - X - COOH Hydrolyzes L-amino acids (including proline) from the C-termini of proteins and peptides. High catalysis rate if the penultimate and/or terminal amino acid carries aromatic or aliphatic side chains. The release of glycine and asparatic acid residues is considerably retarded. Terminal proline and β-Ala are rather good substrates. Dipeptides are completely resistant to cleavage.	Dissolve the content of one vial in 50 μl redist. water. Results in a 50 mM sodium citrate buffer concentration, pH 6.0.	1:100 (protease: protein by weight)
Chymotrypsin Sequencing Grade from bovine pancreas EC 3.4.21.1 M _r = 25 kD	Serine endopeptidase Specifically hydrolyzes peptide bonds at the C-termini of Tyr, Phe, and Trp. Leu, Met, Ala, Asp, and Glu are cleaved at lower rate. Acts upon amides and esters of susceptible amino acids. Specificity is tested with melittin as substrate.	Dissolve in 1 mM HCl.	1: 200 to 1:20 (protease: protein by weight)
Endoproteinase Arg-C Sequencing Grade from <i>Clostridium histolyticum</i> EC 3.4.22.8 M _r = 59 kD	Cysteine protease Specifically hydrolyzes peptide, ester, and amide bonds at the carboxylic side of Arg. Reducing agents (such as DTT) and Ca ²⁺ are required for full activity.	Dissolve the content of one vial enzyme in 50 μl double dist. water to obtain a concentration of 50 mM Tris-HCl buffer, 10 mM CaCl ₂ , 5 mM EDTA, pH 8.0. The activation solution is dissolved in 100 μl double dist. water resulting in a 50 mM DTT and 5 mM EDTA concentration.	1:200 to 1:50 (protease: protein by weight)
Endoproteinase Asp-N Sequencing Grade from <i>Pseudomonas fragi</i> mutant EC 3.4.24.33 M _r = 27 kD	Metallo protease Hydrolyzes peptide bonds at the amino side of Asp and cysteic acid. If cysteine is reduced or alkylated, only - ↓ - Asp-X is cleaved.	Dissolve the content of one vial in 50 μl double dist. water to give a concentration of 10 mM Tris-HCl pH 7.5.	1:200 to 1:20 (protease: protein by weight)
Endoproteinase Glu-C Sequencing Grade from <i>Staphylococcus aureus V8</i> EC 3.4. 21.19 M _r = 27 kD	Serine protease Very specifically hydrolyzes peptide bonds at the carboxylic side of glutamate residues (in ammonium bicarbonate, pH 7.8, or ammonium acetate buffer, pH 4.0), or glutamate and aspartate residues (phosphate buffer, pH 7.8).	Dissolve the enzyme in double dist. water.	1:100 to 1:20 (protease: protein by weight)
Endoproteinase Lys-C Sequencing Grade from <i>Lysobacter enzymogenes</i> EC 3.4. 21.50 M _r = 33 kD (reduced) M _r = 30 kD (non-reduced)	Serine protease Specifically hydrolyzes amide, ester, and peptide bonds at the carboxylic side of lysine.	Dissolve the content of one vial in 50 μl double dist. water to give a concentration of 50 mM HEPES, 10 mM EDTA, 5 mg/ml raffinose, pH 8.0.	1:100 to 1:20 (protease: protein by weight)
Trypsin recombinant, Proteomics Grade from porcine expressed in <i>Pichia pastoris</i> EC 3.4.21.4 M _r = 23.5 kD	Serine endoproteinase Hydrolyzes specifically proteins and peptides at the carboxy side of the basic amino acids arginine and lysine. Amide and ester bonds of arginine and lysine residues are also cleaved.	Make a 0.1 mg/ml solution by adding 250 μl Reconstitution Solution to 25 μg enzyme.	1:100 to 1:20 (protease: protein by weight)

Inhibitor	pH optimum	Application	Purity	Cat. No Pack Size
DFP, PMSF, ZPCK, 4-hydroxymercuribenzoate, and aprotinin	5.5 for acidic, and 7.0 for basic amino acids	Sequence analysis and limited hydrolysis of peptides and proteins, particularly in combination with carboxypeptidase A and B. Determination of C-terminal residues during protein sequencing. Cleaves residues sequentially from the carboxy terminus.	Free of impurities that may interfere with amino-acid analysis. 10 µg Carboxypeptidase Y sequencing grade contains < 10 pmol of each amino acid. Function and purity are checked by amino acid analysis and SDS-PAGE.	11 111 914 001 3 x 20 µg
Aprotinin, DFP, PMSF, phenothiazine-N-carbonyl chloride, TPCK, α ₂ -macroglobulin, α ₁ -antitrypsin, soybean trypsin inhibitor, and chymostatin. No inhibition by APMSF	7.0 to 9.0	Hydrolysis of proteins by chymotrypsin alone or in combination with other proteases. Suitable for peptide mapping, fingerprinting, and sequence analysis.	Free of impurities that may interfere with the specific cleavage or separation of peptides in reverse phase HPLC.	11 418 467 001 4 x 25 µg
Oxidizing agents, sulfhydryl reactants (e.g. TLCK), EDTA, Co ²⁺ , Cu ²⁺ , Cd ²⁺ , citrate, borate, and Tris anions partially inhibit	7.2 to 8.0	Specific cleavage of proteins and peptides for peptide mapping, fingerprinting, and sequence analysis. Digestion of proteins in solution, in gels or on membranes.	Free of impurities that may interfere with the specific cleavage or separation of peptides in reverse phase HPLC.	11 370 529 001 3 x 5 µg
EDTA and α-phenanthroline	7.0 to 8.0	Protein-structure and sequence analysis. For protein sequencing or sequence verification, analysis of protein structural domains and cleavage of fusion proteins.	Free of impurities that may interfere with the specific cleavage or separation of peptides in reverse phase HPLC. Purity is checked by SDS-PAGE and silver staining.	11 420 488 001 2 µg 11 054 589 001 3 x 2 µg
DFP, α ₂ -macroglobulin, and TLCK	4.0 to 7.8	Protein-structure and sequence analysis.	Free of impurities that may interfere with the separation of peptides in reverse phase HPLC.	11 420 399 001 50 µg 11 047 817 001 3 x 50 µg
DFP, TLCK, aprotinin, and leupeptin	8.5 to 8.8	Protein-structure and sequence analysis. Suited for the digestion of proteins in polyacrylamide gels. For protein sequencing or sequence verification, analysis of protein structural domains and cleavage of fusion proteins.	Free of impurities that may interfere with the separation of peptides in reverse phase HPLC. Purity is checked by HPLC and SDS-PAGE using silver staining.	11 420 429 001 5 µg 11 047 825 001 3 x 5 µg
TLCK, DFP, PMSF, leupeptin, soybean trypsin inhibitor, trypsin inhibitor from hen egg, aprotinin, α ₂ -macroglobulin, α ₁ -antitrypsin, APMSF, and antipain	8.5 to 8.5	Especially designed for the digestion of proteins separated by 2D gel electrophoresis or liquid chromatographic methods in the course of sample preparation. Qualified for use with in-gel digestion and mass spectrometric analysis.	Free of activity from other proteases, especially chymotrypsin which is absent from the formulation < 95% (gel filtration).	03 708 985 001 4 x 25 µg 03 708 969 001 4 x 100 µg

Mass Spectrometry – Data

Clear identification of peptides

Lysozyme digested with Endoproteinase Asp-N Sequencing Grade (16 hours incubation time)

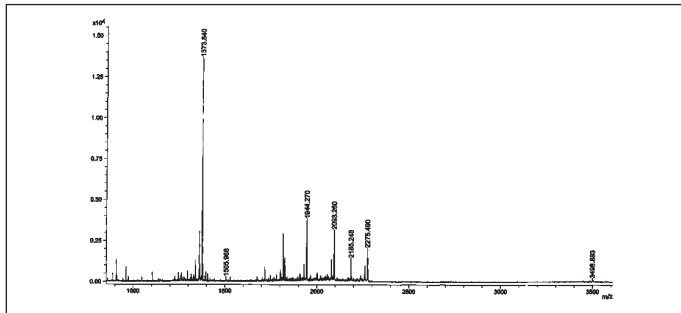


Figure 3: Endoproteinase Asp-N Sequencing Grade digest conditions.

1 mg/ml solution of lysozyme was reduced with 1,4 dithiothreitol and alkylated with iodoacetamide. To show the high enzyme stability the lysozyme was digested with Endoproteinase Asp-N Sequencing Grade previously incubated at 25°C for 24 hours using a 1:100 enzyme: protein ratio in 50 mM sodium phosphate buffer pH 8.0. The digest was incubated at 37°C for 16 hours. The sample was desalted using a ZipTip[®]_{μ-C18} pipette tip from Millipore and eluted directly onto the MALDI target using the MALDI matrix (alpha-cyano-4-hydroxycinnamic acid, 10 mg/ml in 70% ACN, 0.03% TFA). MALDI analysis was performed in the reflector positive ion mode with a Reflex III instrument (Bruker).

Peak	Amino Acid	Missed Cleavages	(M+H) ⁺
P1	119 - 129	0	1373.84
P2	87 - 100	0	1505.97
P3	1 - 17	0	1944.27
P4	101 - 118	0	2093.26
P5	48 - 65	1	2185.25
P6	66 - 86	0	2275.49
P7	18 - 47	0	3498.89

Sequence coverage: 100%

Sequence coverage with other proteases:

Trypsin, Proteomics Grade: 95%;

Endoproteinase Lys-C Sequencing Grade: 100%;

Endoproteinase Arg-C Sequencing Grade: 100%

rGFP digested with Trypsin, Proteomics Grade (16 hours incubation time)

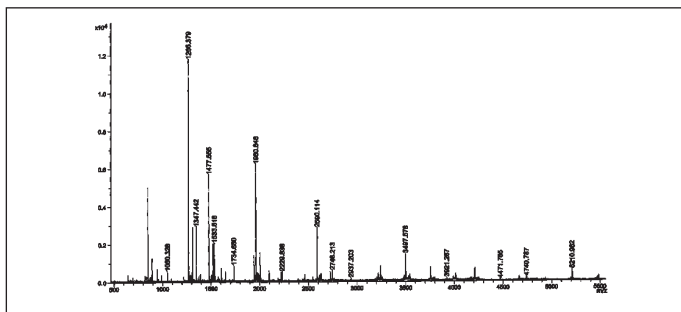


Figure 4: Trypsin, Proteomics Grade digest conditions.

1 mg/ml solution of rGFP was reduced with 1,4 dithiothreitol and alkylated with iodoacetamide. The rGFP was digested with Trypsin, Proteomics Grade at a 1:100 enzyme: protein ratio in 25 mM Tris, 1 mM EDTA, 2 M UREA, pH 8.5. The digest was incubated at 25°C for 16 hours. The sample was desalted using a ZipTip[®]_{μ-C18} pipette tip from Millipore and eluted directly onto the MALDI target using the MALDI matrix (alpha-cyano-4-hydroxycinnamic acid, 10 mg/ml in 70% ACN, 0.03% TFA). MALDI analysis was performed in the reflector positive ion mode with a Reflex III instrument (Bruker).

Peak	Amino Acid	Missed Cleavages	(M+H) ⁺
P1	114 - 122	0	1050.33
P2	86 - 96	0	1266.38
P3	97 - 107	1	1347.44
P4	110 - 122	1	1477.55
P5	114 - 126	1	1533.62
P6	127 - 140	1	1542.59
P7	97 - 109	2	1604.61
P8	108 - 122	2	1734.68
P9	110 - 126	2	1960.85
P10	141 - 156	0	1973.65
P11	141 - 158	1	2229.84
P12	216 - 238	0	2590.11
P13	215 - 238	1	2746.21
P14	132 - 156	1	2937.20
P15	127 - 156	2	3497.58
P16	4 - 41	1	3921.27
P17	169 - 209	0	4471.79
P18	167 - 209	1	4740.79
P19	169 - 215	2	5210.96

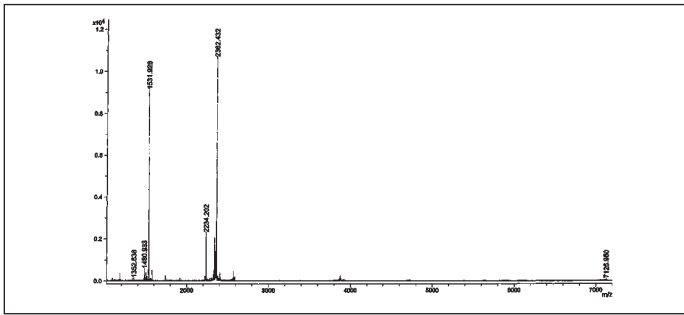
Sequence coverage: 77%

Sequence coverage with other proteases:

Endoproteinase Lys-C Sequencing Grade: 78%

Endoproteinase Asp-N Sequencing Grade: 72%

Lysozyme digested with Endoproteinase Lys-C Sequencing Grade (1 hour incubation time)



Peak	Amino Acid	Missed Cleavages	(M+H) ⁺
P1	2 - 13	0	1352.84
P2	1 - 13	1	1480.93
P3	117 - 129	0	1531.93
P4	98 - 116	0	2234.2
P5	14 - 33	0	2337.28
P6	97 - 116	1	2362.43
P7	34 - 96	0	7125.95

Sequence coverage: 100%

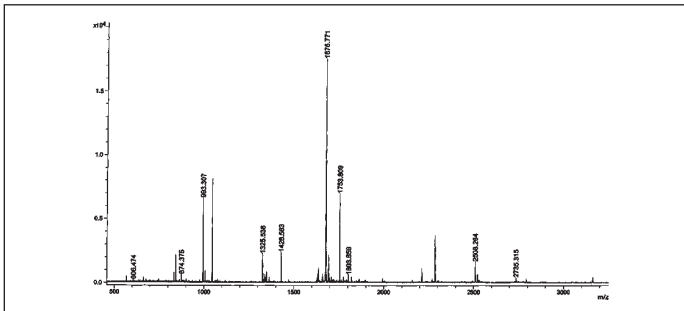
Sequence coverage with other proteases:

Trypsin, Proteomics Grade: 100%

Figure 5: Endoproteinase Lys-C Sequencing Grade digest conditions.

1 mg/ml solution of lysozyme was reduced with 1,4 dithiothreitol and alkylated with iodoacetamide. The lysozyme was digested with Endoproteinase Lys-C Sequencing Grade at a 1:100 enzyme: protein ratio in 25 mM Tris, 1 mM EDTA, 2 M UREA, pH 8.5. The digest was incubated at 37°C for 1 hour. The sample was desalted using a ZipTip®_{μ-C18} pipette tip from Millipore and eluted directly onto the MALDI target using the MALDI matrix (sinapic acid, 10 mg/ml in 70% ACN, 0.03% TFA). MALDI analysis was performed in the reflector positive ion mode with a Reflex III instrument (Bruker).

In gel digest of Lysozyme with Trypsin, Proteomics Grade (16 hours incubation time)



Peak	Amino Acid	Missed Cleavages	(M+H) ⁺
P1	1 - 5	1	606.47
P2	15 - 21	0	874.37
P3	62 - 68	0	993.31
P4	22 - 33	0	1325.54
P5	115 - 125	1	1333.56
P6	34 - 45	0	1428.58
P7	98 - 112	0	1675.77
P8	46 - 61	0	1753.81
P9	97 - 112	1	^ 1803.86
P10	74 - 96	0	2508.26
P11	22 - 45	1	2735.32

Sequence coverage: 84%

Sequence coverage with other proteases:

Endoproteinase Lys-C Sequencing Grade: 84%;

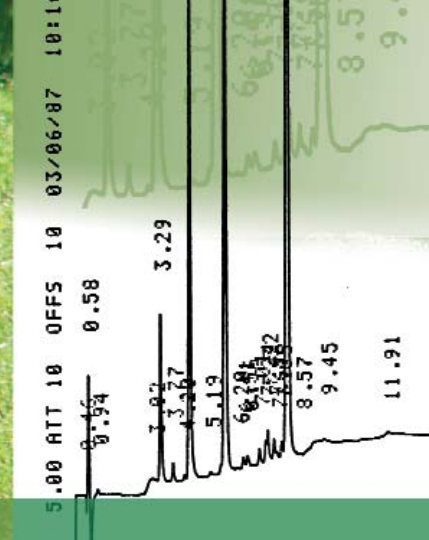
Endoproteinase Asp-N Sequencing Grade: 100%;

Endoproteinase Arg-C Sequencing Grade: 98%

Figure 6: In Gel Trypsin, Proteomics Grade digest conditions.

The coomassie stained protein band was discolored with 0.1 M ammoniumhydrogencarbonat in 30% acetonitril, reduced with 1,4 dithiothreitol and alkylated with iodoacetamide. The protein band was digested with Trypsin, Proteomics Grade at 0.28 ug enzyme per band in 2 mM Tris, pH 8.5. The digest was incubated at 25°C for 16 hours in the dark. The protein was extracted in 20 % acetonitril, 0.1% TFA and 1.3 mM Tris. The sample was desalted using a ZipTip®_{μ-C18} pipette tip from Millipore and eluted directly onto the MALDI target using the MALDI matrix (alpha-cyano-4-hydroxycinnamic acid, 10 mg/ml in 70% ACN, 0.03% TFA). MALDI analysis was performed in the reflector positive ion mode with a Reflex III instrument (Bruker).

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Related Products

Characterize the nature of glycans

- Glycosylation is one of the most common post-translational modifications of proteins in eukaryotic cells. Sugar structures cannot be predicted from a genome sequence, so must be analyzed directly from glycoproteins.
- Use the DIG Glycan Detection Kit to help determine whether or not the sample contains glycoproteins.
- Work with Endo- and/or Exoglycosidases to find out if the side chains are N- or O-linked and what type of N-linked chain is present.
- Use the DIG Glycan Differentiation Kit to characterize and analyse which sugars occur in the glycoprotein side chains.

Endoglycosidases	Cat. No.	Pack Size
α -Amylase from pig pancreas	10 102 814 001	50 mg (5 ml)
Endoglycosidase H from <i>Streptomyces plicatus</i> , rec. from <i>E. coli</i>	11 088 726 001 11 643 053 001	1 U (200 μ l) 2.5 U (500 μ l)
N-Glycosidase A PNGase A from almond	11 642 995 001	5 mU (0.1 ml)
O-Glycosidase from <i>Diplococcus pneumoniae</i>	11 347 101 001	25 mU
N-Glycosidase F, lyophilizate PNGase F of <i>Flavobacterium meningosepticum</i> , rec. from <i>E. coli</i>	11 365 185 001 11 365 193 001	100 U 250 U
N-Glycosidase F, solution PNGase F of <i>Flavobacterium meningosepticum</i> , rec. from <i>E. coli</i>	11 365 169 001 11 365 177 001	100 U (0.1 ml) 250 U (0.25 ml)
N-Glycosidase F Deglycosylation Kit	11 836 552 001	1 Kit
Hyaluronidase from ovine testes	10 106 500 001	100 mg
Lysozyme Muramidase from hen egg white	10 837 059 001	10 g
Kits for Glycan Analysis	Cat. No.	Pack Size
DIG Glycan Detection Kit	11 142 372 001	1 Kit
DIG Glycan Differentiation Kit	11 210 238 001	1 Kit

Exoglycosidases	Cat. No.	Pack Size
Amyloglucosidase special quality for starch determination, from <i>Aspergillus niger</i>	11 202 332 001 11 202 367 001	500 U 3,500 U
Amyloglucosidase from <i>Aspergillus niger</i>	10 102 857 001	100 mg (10 ml)
β -Fructosidase from yeast	10 104 922 011	750,000 U
β -Galactosidase from <i>E. coli</i> overproducer	10 105 031 001	1,500 U
β -Galactosidase from <i>E. coli</i> overproducer, EIA grade	10 567 779 001 10 745 731 001	5 mg 25 mg
β -Glucuronidase from <i>E. coli</i> K 12	03 707 580 001 03 707 598 001 03 707 601 001	1 ml 5 ml 15 ml
Neuraminidase (Sialidase) from <i>Arthrobacter ureafaciens</i>	10 269 611 001	1 U (100 μ l)
Neuraminidase (Sialidase) from <i>Clostridium perfringens</i> , special quality with low protease content	11 585 886 001	5 U
Neuraminidase (Sialidase) from <i>Vibrio cholerae</i>	11 080 725 001	1 U



Diagnosics

For details on products and application, please visit the protein analysis section via our homepage <http://www.roche-applied-science.com>

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