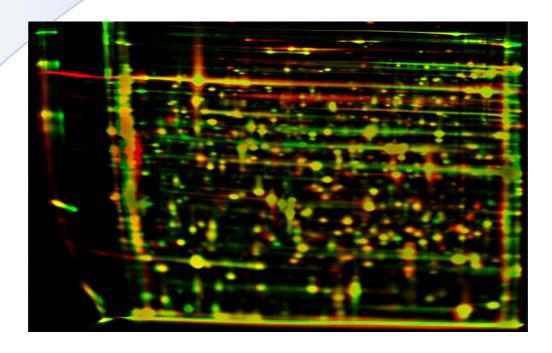
NEW!

Trypsin NB Premium Grade, MS approved Trypsin NB Sequencing Grade, modified

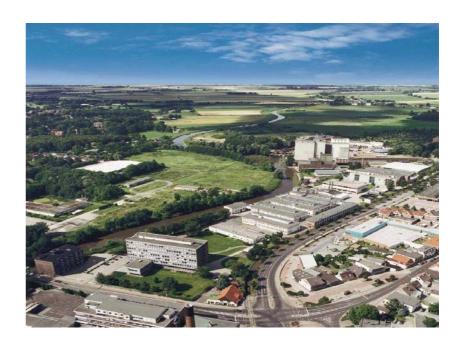


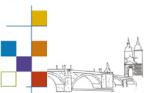




SERVA Trypsins for use in Proteomics

Produced by pharmaceutical company Nordmark GmbH & Co. KG in Germany

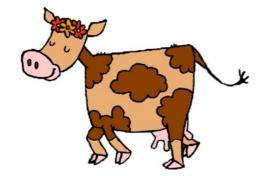


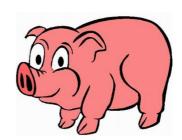




What is trypsin?

- ➤ It is a serine endopeptidase which <u>specifically</u> cleaves peptide bonds at the carboxyl side of lysine, arginine and S-amino-ethyl cysteine residues. There is little or no cleavage at arginyl-proline or lysyl-proline bonds.
- ➤ It is isolated from pancreas (porcine or bovine) and purified by chromatography



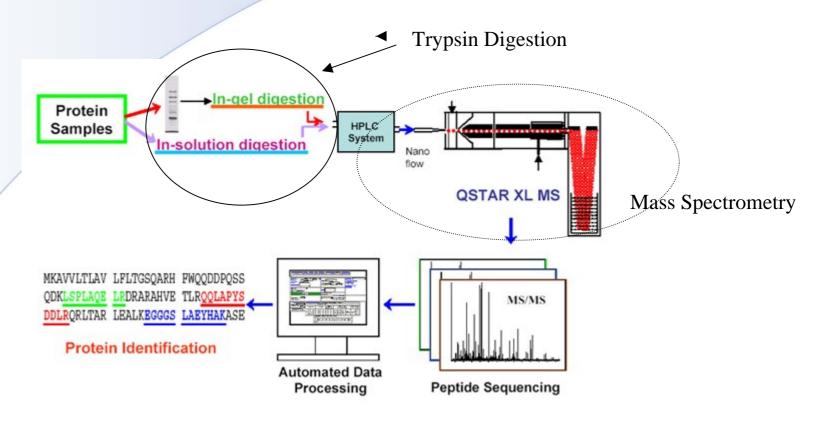






Used for what?

For digestion of proteins prior to sequence analysis for proteomics investigations







Main competitors on the market:

Roche

Trypsin sequencing grade (not modified) (cat.no. 11 418 025 001)

Trypsin modified, sequencing grade (cat.no. 1 418 475)

Promega

Sequencing Grade Modified Trypsin (cat.no.V5111)

Trypsin Gold, Mass Spectrometry Grade (cat.no.V5280)





Trypsins for use in Proteomics

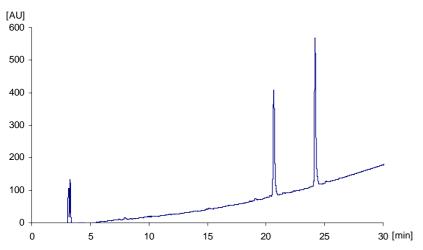
Good protein digestion to peptides is a prerequisite for exact protein identification. To obtain reproducible, reliable results and a high significance of searched results (Mascot Score) the following features are of importance:

- > Specificity
- > Purity
- Absence of chymotryptic activity
- > Stability
- > Low autoproteolyis





High Specificity



Specificity of Trypsin NB Premium Grade, MS approved analyzed by Reversed Phase HPLC. RP Fragments: 20.6 min Gly (23)-Lys (29), 24.2 min Phe (1)-Arg (22)

The specificity of SERVA Trypsins is verified with the oxidized B chain of insulin (insulin B_{ox}) as substrate.

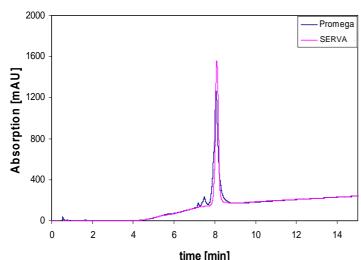
25 μ g of insulin B_{ox} are incubated with 0.5 μ g Trypsin NB Premium Grade, MS approved at 37 °C for 18 h to detect traces of impurities of chymotrypsin.

- > Specific fragments
- No artefacts
- Prerequisite for protein quantification and indentification





- High Purity
- Absence of chymotryptic activity



Purity of Trypsin Sequencing Grade, modified and Promega Trypsin Gold, Mass Spectrometry Grade in reversed phase HPLC.

SERVA Trypsins are highly purified enzyme preparation that are free of activity from other proteases. The absence of chymotryptic activity is verified by purity and function control which is carried out for each lot.



- > Specific fragments
- High stability





High Stability during performance

Incubation time (h)	Activity (%)			
	SERVA Trypsin NB	Trypsin native, not modified		
0	100	100		
3	100	43		
5	87	30		
7	84	25		
22	46	5		

Stability of Trypsin NB Sequencing Grade modified and Trypsin native, not modified in 20 mM Tris- HC, pH 8.0 at 37 °C

SERVA Trypsins are modified by reductive methylation and purified by chromatography, yielding a highly active molecule that is extremely resistant to autolytic digestion.



- > High activity
- High resistance to autolytic digestion





- High stability during storage
 - Lyophilised:
 - 2-8 °C , 12 month
 - - 20 °C, 12 month (ongoing)
 - Solubilized:
 - 2 -8 °C: 4 weeks
 - -20 °C: 4 weeks
 - 4 freeze and thaw cycles



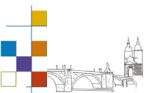
convenient preparation of aliquots





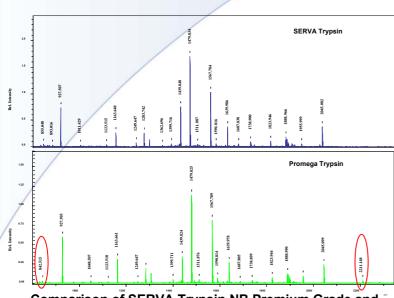
- High stability during transportation
 - Lyophilised:
 - 40 °C, 7 days

No need of dry ice during transportation saves costs saves space





Exceptionally low autoproteolysis



Comparison of SERVA Trypsin NB Premium Grade and Promega Trypsin Gold. Spectra of BSA digested with SERVA Trypsin NB Premium Grade, MS approved and Promega Trypsin Gold, Mass Spectrometry Grade are shown. Trypsin masses are indicated

Based on excellent and proprietary production procedures, SERVA Trypsins exhibit exceptionally low autocatalytic activity resulting in unique stability. Nearly any trypsin peptides can be detected in the reaction mixture after standard digestion of single proteins or complex protein mixtures.

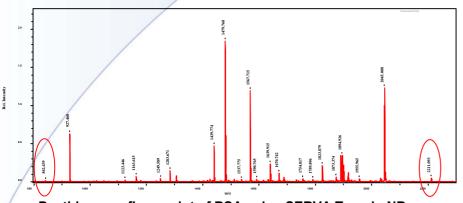
- High stability
- > No interferring masses
- > Easy interpretation of spectra





Trypsin Peptide (TP) Standard

- contains trypsin to generate masses m/z 842 and 2211
- is delivered with the SERVA Trypsins



Peptide mass fingerprint of BSA using SERVA Trypsin NB Premium Grade and TP Standard in a ratio of 5:1.

BSA digestion was carried out with SERVA Trypsin NB Premium Grade, MS approved and TP Standard. Desired masses of m/z 842 and 2211 are indicated.

Some user (especially those who work with MALDI as ionisation) need standard peptides for internal calibration.

TP Standard facilitates easy internal calibration to enhance mass accuracy in MS analysis and can be adjusted to any experimental conditions.



- > easy internal calibration
- adjustable to any experimental conditions





Trypsin NB Sequencing Grade, modified CoA



CERTIFICATE OF ANALYSIS

Trypsin NB Sequencing Grade, modified from porcine pancreas		Cat. No. : Lot. No. :	37283 070119
Parameter	Method	Specification	Result
Sequence specificity	Digest of Insulin B chain (ox.) HPLC analysis	Must comply	Complies

For customer information:

Origin Porcine pancreas Molecular weight 24 kDa (trypsin) Appearance White to off-white powder

Purity (HPLC) > 90%

> 6.000 U/g lyophilisate Tryptic activity

01/2008 Minimum shelf life Storage conditions -15 to -25 °C

Detection of two distinct peaks in Reversed Phase HPLC after digestion of oxidized B-chain of insulin at 37°C for 18h.

Unit definition:

Tryptic activity:

1 Unit catalyzes the hydrolysis of 1 μ mol N α -Benzoyl-L-arginine -4-nitroanilide hydrochloride (BAPNA) per minute at 30 °C, pH 8.0.





Trypsin NB Premium Grade, MS approved CoA



CERTIFICATE OF ANALYSIS

Each lot is qualified by in-gel digestion and mass spectrometric analysis

Trypsin NB Premium Grade, MS approved from porcine pancreas		Cat. No. : Lot. No. :	37284 080171
Parameter	Method	Specification	Result
Sequence specificity	Digest of Insulin B chain (ox.) HPLC analysis	Must comply	Complies
Application (MS) approval	In-gel digestion of BSA and Mass spectrometric analysis	Must comply	Complies

For customer information:

 Origin
 Porcine pancreas

 Molecular weight
 24 kDa (tryptim)

 Appearance
 White to off-white powder

 Purity (HPLC)
 > 90%

 Tryptic activity
 > 6.000 U/g lyophilisate

 Minimum shelf life
 02/ 2009

 Storage conditions
 -15 to -25 °C

Sequence specificity assay:

Detection of two distinct peaks in Reversed Phase HPLC after digestion of oxidized B-chain of insulin at 37°C for 18h.

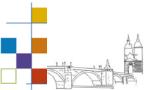
Application (MS) approval:

In-gel digest and identification of BSA.

Unit definition:

Tryptic activity:

1 Unit catalyzes the hydrolysis of 1 timol Not-Benzoyl-L-arginine -4-nitroanilide hydrochloride (BAPNA) per minute at 30 °C, pH 8.0.





Marketingmaterial

- > Flyer
- Product Information
- User Manual
- > Poster
- > Samples





TECHNICAL NOTES SERVA

Blochemicals

Electrophoresis

Bloseparation Life Sciences Specials

Product Description

Trypsin NB Premium Grade, MS approved is designed for digestion of proteins prior to sequence analysis. Each lot is qualified for use with in-gel digestion and mass spectrometric analysis. Based on excellent and proprietary production procedures, Trypsin NB Premium Grade, MS approved is of unique stability due to exceptionally low autocatalytic activity.

Trypsin NB Premium Grade, MS approved is a serine endopeptidase which specifically cleaves peptide bonds at the carboxyl side of lysine, arginine and S-aminoethyl cysteine residues. There is little or no cleavage at arginyl-proline or lysyl-proline bonds. Cleavage may also be considerably reduced when acidic residues are present on either side of a potentially susceptible

Outstanding performance is guaranteed by:

- · Each lot MS approved
- · Exceptionally low autoproteolysis
- · Extreme stability
- High purity High specificity
- · No chymotryptic activity

Trypsin NB Premium Grade, MS approved is supplied as lyophilisate in vials at 25 µg each. It is manufactured by the pharmaceutical plant of Nordmark Arzneimittel GmbH & Co. KG. Germany.

Incubation time (h)	Activity (%)		
	Trypsin NB Premium Grade, MS appoved	Trypsin native, not modified	
0 3 5 7	100 100 87 84	100 43 30 25	
22	46	5	

Tab.1: Stability of Trypoin NB Premium Grade, MS approved and Trypoin native, not modified in 20 mM Trip-HCl, pH 8.0 at 37 °C.

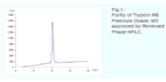
Trypsin NB Premium Grade, MS approved

Extreme Stability

Trypsin NB Premium Grade, MS approved is modified by reductive methylation and purified by chromatography, yielding a highly active molecule that is extremely resistant to autolytic digestion (Tab. 1).

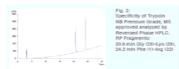
High Purity

Trypsin NB Premium Grade, MS approved is a highly purified enzyme preparation that is free of activity from other proteases. The absence of chymotryptic activity is verified by purity and function control which is carried out for each lot (Fig. 1, Fig. 2).



High Specificity

The specificity of Trypsin NB Premium Grade, MS approved is verified with the oxidized B chain of insulin (insulin B.,) as substrate, 25 µg of insulin B., are incubated with 0.5 µg Trypsin NB Premium Grade, MS approved at 37 °C for 18 h to detect traces of impurities of chymotrypsin (Fig. 2).



TECHNICAL NOTES

Electrophoresis

Trypsin NB Premium Grade, MS approved

Quality Control

Each lot of Trypsin NB Premium Grade, MS approved is qualified by in-gel digestion and mass spectrometric analysis. An example of a spectrogram is shown in figure 3. Lot specific generated spectrograms using bovine serum albumin (BSA) as substrate are available at tech service@serva.de.

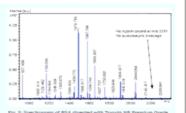


Fig. 3: Spectrogram of BSA digeoted with Trypoln NB Premium Grade MS approved, 300 ng BSA were deparated by gel electrophoreolo and digeoted with 10 nglyl Trypnin NB Fremium Grade, MB approved in 50 mM NH, HCO, at 37 °C overnight. The peptides generated were analysed in reflectron mode using the Bruker Ultraffex MALCHTOF/TOF maso opectrometer, indicated maso values were identified as BSA protein upling the Maccot bearch engine (Score>300). No bignate modified trypolog exhibited autocatalytical activity under identical known tryolin peptide. Macco coreo for protein identification were olgnificant higher uoing Tryolin NB Premium Grade, MS approved than for other modified trypolno (Ref: A. Pich, unpublished, Medical

Contact us!

If you require more detailed information, please contact us:

Technical Support

Pinnauallee 4 · D-25436 Uetersen Tel.: +49-4122-712534 E-Mail: tech.service@serva.de

Features at a glance:

- · Source: porcine pancreas
- Purity: > 90 %
- Tryptic activity: > 6000 U/g*
- · No chymotryptic activity detectable
- · Modified by reductive methylation
- Each lot qualified by in-gel digestion and mass
- spectrometric analysis

Convenient packing: 4 vials at 25 μg each

*Unit definition: 1 U catalyzes the hydrolysis of 1 µmol Na-Benzovi

Ordering Information

Product	Quantity	CatNo.
Trypsin NB Premium Grade, MS approved	4 x 25 μg	37284.01

Related Products

Product	Quantity	CatNo.
Trypsin NB Sequencing Grade, modified	4x25 μg	37283.01
Trypsin NB Sequencing Grade	4x25 μg	37280,01
Endoproteinase Arg-C	3х5µд	20960.01
Endoproteinase Glu-C	1 mg	20985.01

(1) Wilkinson, J. M. (1898): Fragmentation of Polypeptides by Enzymatic Methodo, In: Practical Protein Chemistry: A Handbook, A. Darbre, ed., John Wiley and Sono, New York, N.Y.









SERVA

PRODUCT INFORMATION

Trypsin NB Premium Grade, MS approved

Cat.No. 37284

Product Description:

General

Trypsin NB Premium Grade, MS approved is a serine endopeptidase which specifically cleaves at the carboxyl side of lysine, arginine and S-aminoethy dystein ersidues. There is little or no dieavage at arginyl-proline or lysyl-proline bonds. Cleavage may also be reduced when addit or ensidues are present on either side of a potentially susseptible bond (1).

Application

Trypsin NB Premium Grade, MS approved is specially designed for digestion of proteins prior to mass spectrometric analysis.

- Source: poroine pancreas
- Purity: > 90 %
- Tryptic activity: > 6000 U/g*
- No chymotryptic activity detectable
 Modified by reductive methylation
- Each lot qualified by in-gel digestion and mass spectrometric
- Quantity: ≥ 25 µg/vial, determined by measuring A₂₈₀.
- "Unit definition: 1 U catalyzes the hydrolysis of 1 µmol No-Benzoyl-Larginine-4-nitroanilide hydrochloride (BAPNA) per minute at 30 °C, pH 8 0

Specificity

The specificity of Trypsin NB Premium Grade, MS approved is verified with oxidized 8 chain of insulin insulin ing. as substrate: 25 ug insulin, are incubated with 0.5 ug Trypsin NB Premium Grade, MS approved, dissolved in 100 mM Tris-HCl buffer, pH 8.5 in a total volume of 25 µl at 37 °C for 18 h to detect traces of impurities of chymotrypsin (figure 1).



Fig. 1: Specificity of Trypsin NB Premium Grade, MG approved analyzed by Reversed Phase HPLC, RP HPLC is performed under following conditions: Column PepMagn of 18⁴. Solvent A. 0, 1.5 17 A. (vv.) in aster, Solvent B. 0, 1% TFA (viv.) in adecidinine, Gradeett, 30 min linear 0 - 55 %. B (fill of table 1 minms, Vava length, 2 15 min. Pragments, 206 min (v) (23) - Lys

Version 01/5

DERIVA Electrophoresis GmbH = D-69115 Heidelberg = Carl-Benz-Str. 7

Phone + 49 (C) 6221 / 13840-0 = Pax = 49 (O) 6221 / 13840-10 = email info@serva.de = http://www.serva.de



PRODUCT INFORMATION

Trypsin NB Sequencing Grade, modified

Cat.No. 37283

Product Description:

Genera

Trypsin NB Sequencing Grade, modified is a serine endopeptidase which specifically cleaves at the carboxyl side of fysine, arginne and S-aminoethyl cysteine residues. There is little or no cleavage at arginyl-proline or lysyl-proline bonds. Cleavage may also be reduced when acidio residues are present on either side of a potentially susceptible bond (1).

Application

Trypsin NB Sequencing Grade, modified is specially designed for digestion of proteins prior to mass spectrometric analysis.

Features

- Source: porcine pancreas
- Purity: > 90 %
- Tryptic activity: > 6000 U/g*
- No chymotryptic activity detectable
- Modified by reductive methylation
 Quantity: 2.25 µg/vial, determined by measuring A₂₆₀.

"Unit definition: 1 U catalyzes the hydrolysis of 1 µmol No-Benzoyl-L-

arginine-4-nitroanilide hydrochloride (BAPNA) per minute at 30 °C, pH 8.0.

Stability

Trypsin NB Sequencing Grade, modified is more resistant towards autolysis even at pH values in weakly basic range (table 1). Therefore the enzyme can be used in high concentrations in the digestion assay.

incubation time (h)	Activity (%)		
	Trypsin NB Sequencing Grade, modified (Cat. No. 97285)		
0	100	100	
3	100	43	
6	87	30	
7	84	25	
22	46	5	

Tab. 1: Stability of Trypsin N8 Sequencing Grade, modified and Trypsin native, not modified in 20 mM Tris-HCl, pH 8.0 at 37 °C

Storage	Trypsin NB Sequencing Grade, modified should be stored in a dry
conditions	state at -15 to -25 °C.

Version 01/00

SERVA Electrophoresis GmbH • D-69115 Heidelberg • Carl-Benz-Str. 7

Phone • 49 (0) 6221 / 13840-0 • Fax • 49 (0) 6221 / 13840-10 • email info@serva.de • http://www.serva.de



User Protocol

Trypsin NB Sequencing Grade, modified Cat.No. 37283
Trypsin NB Premium Grade, MS approved Cat.No. 37284

Product Description:

General

SERVA Trypsins (Cat.No. 37283, 37284) are specially designed for the digestion of proteins prior to mass spectrometric analysis. Trypsin is a serine endopeptidases which specifically cleaves at the carboxyl side of lysine, arginine and S-aminoethyl cysteine residues. There is little or no cleavage at arginyl-proline or lysyl-proline bonds. Cleavage may also be considerable reduced when acidio residues are present on either side of a potentially suspectible bond [11].

SERVA Trypsins (Cat.No. 37283, 37284) have been produced to provide maximum performance. They are manufactured by the pharmaceutical plant of Nordmark Arzneimittel GmbH & Co. KG in Germany.

Features

- · Source: porcine pancreas
- Purity: > 90 %
- Tryptic activity: > 6000 U/g*
- · No chymotryptic activity detectable
- · Verified specificity
- Quantity: ≥ 25 µg/vial, determined by measuring A₂₈₀.
- Modified by reductive methylation (Cat.No. 37283, 37284)
- Each lot qualified by in-gel digestion and MS analysis (Cat.No. 37284)

For further details please see product information available at www.serva.de.

"Unit definition: 1 U catalyzes the hydrolysis of 1 µmol No-Benzoyi-Larginine-4-nitroanilide hydrochloride (BAPNA) per minute at 30 °C, pH 8.0.

Storage conditions

Store lyophilisate in a dry state at -15 to -25 °C

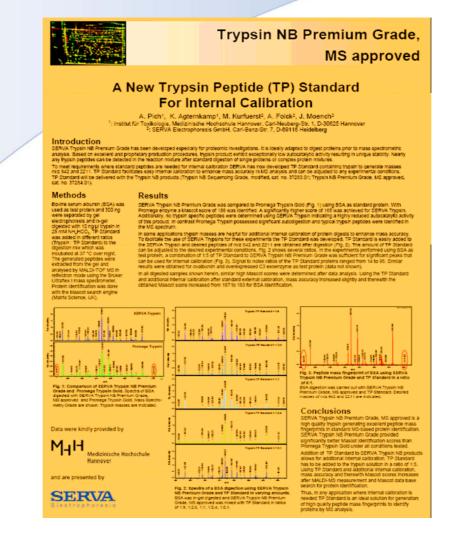
 Wilkinson, J. M. (1986): Fragmentation of Polypeptides by Enzymatic Methods. In: Practical Protein Chemistry: A Handbook, A. Darbre, ed., John Wiley and Sons, New York, N.Y.

DERVA Electrophoresis GmbH = 0-69115 Heldelberg = Carl-Benz-6tr, 7

Fhone + 49 (0) 6221 / 13840-0 = Fax + 49 (0) 6221 / 13840-10 = email info@serva.de = http://www.serva.de











SERVA Trypsins vs. Promega Trypsins

SERVA

Promega

	Trypsin NB Premium Grade, MS approved	Trypsin NB Sequencing Grade, modified	Trypsin Gold, Mass Spectrometry Grade	Sequencing Grade Modified Trypsin
Stability (solubilized) 4 °C, -20 °C	4 weeks, 4 weeks	4 weeks, 4 weeks	-, 4 weeks	-, 4 weeks
Storage Stability (lyophilized) 40 °C, 4 °C, -20 °C	7 days, 1year, 1year (ongoing)	7 days, 1year, 1year (ongoing)	3 days 57 °C*, 11/2 year *	3 days 57 °C*, 11/2 year *
Shipping conditions	RT	RT	Dry ice	Dry ice
Qualified by MS analysis	✓		✓	
Autoproteolytis	no tryptic masses	no tryptic masses	tryptic masses	tryptic masses
Efficacy	✓ ✓	✓ ✓	✓	✓
Purity	✓ ✓	✓ ✓	✓	✓
Mascot Score with BSA as reference	✓ ✓	✓ ✓	✓	✓
Package size	4 vials each 25 μg + TP	4 vials each 25 μg + TP	1 vial, 100 μg	1 vial, 100 μg
Source	porcine	porcine	bovine	bovine



Package size:

4 vials, each 25 μg lyophilisate + TP Standard

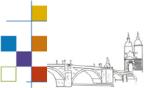
CatNo.	Product	Quantity	Price (net)
37283.01	Trypsin NB Sequencing Grade	4 vials each 25 μg + TP	55,30 €
37284.01	Trypsin NB Premium Grade, MS approved	4 vials each 25 μg +TP	59.50 €



- Convenient package size
- Protection against contamination

Sample size:

1 vial each 25 μg + TP Standard





Promega Price structure in EU

	France/ BeNeLux	Italy	Spain	GB	Sweden
Sequencing Grade Modified Trypsin (1 vial, 100 µg)	73€	77 €	81 €	47 £	829 SEK
Trypsin Gold, Mass Spectrometry Grade (1 vial, 100 µg)	102 €	112 €	112 €	55 £	885 SEK

SERVA	Quantity	Price (net)
Trypsin NB Sequencing Grade	4 vials each 25 μg + TP	55,30 €
Trypsin NB Premium Grade, MS approved	4 vials each 25 μg +TP	59.50 €



Good price situation for product launch





Trypsin NB Premium Grade, MS approved Trypsin NB Sequencing Grade, modified - Outstanding Characteristics

- Due to high efficacy and high purity SERVA
 Trypsins are applicable for quantitative analysis
- Nearly any autoproteolytic activity leads to very reduced tryptic background
- TP standard allows internal calibration and adaption to any experimental conditions
- Better significance of searched results than for Promega
- Shipping at RT instead on dry ice reduces costs
- Competative price



