

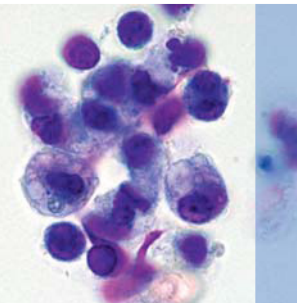
Biochemicals  
Electrophoresis  
Bioseparation  
Life Sciences  
Specials

# Collagenase NB

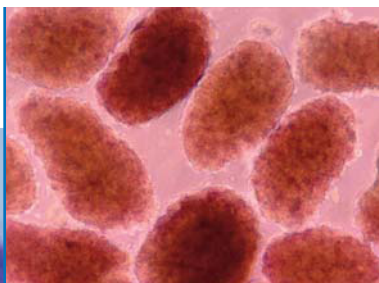
## Application Details for Tissue Dissociation

### Collagenase NB from SERVA Electrophoresis

Collagenases from *Clostridium histolyticum* are proteolytic enzymes that cleave peptide bonds in the triple helical collagen molecule of human or animal tissue *in situ*. **Nordmark Collagenase NB** qualities are particularly suitable for cell isolation from various tissues and passaging of stem cells. The application of pharmaceutical manufacturing standards guarantees stringent quality control, reliable lot-to-lot consistency and excellent performance of the product. TSE safety of the fermentation product has been certified by the European authority EDQM and by supplier's certificate of origin.



Human adipocytes, by courtesy of Pharmicell Europe GmbH, Germany



Human islets of Langerhans, by courtesy of H. and D. Brandhorst, Uppsala University Hospital, Sweden

### SERVA GMP Grade enzymes

Enzymes for tissue dissociation are critical raw materials for tissue engineering and transplantation into humans. Thus Nordmark offers superior GMP grade Collagenase NB and Neutral Protease NB qualities that are manufactured in compliance with the EU-Guide to current Good Manufacturing Practice. Furthermore, TSE safety of the products is certified. Virus validation studies and, for each lot, testing for abnormal toxicity (according European Pharmacopoeia) are performed.

### Storage and reconstitution

All Collagenase NB products are provided as lyophilized powders and can be stored at +2 to +8 °C. They remain stable without loss of activity for at least one year if protected from moisture. Collagenase NB qualities are soluble in common buffers, e. g. HBSS. Reconstituted enzymes can be sterile filtered, aliquoted and stored at -20 °C for one year. Repeated freezing and thawing should be avoided.

### Optimization of tissue digestion

In general the required collagenase concentration depends on tissue type and origin as well as isolation procedure. Therefore Collagenase NB concentrations stated in the following table should be considered as starting points and progress of the digestion process should be monitored visually in order to determine the optimal conditions. Collagenase activity is stated in PZ units according to Wunsch and shows an optimum at 37 °C and at pH ~7.4. As collagenase requires calcium for full catalytic activity 2 mM Ca<sup>2+</sup> is recommended.

### Inactivation and inhibitors

Digestion progress can be reduced by cooling down or dilution of the digestion solution. Collagenase is reversibly inactivated at high pH values and irreversibly inactivated at low pH values. Inhibitors of collagenase are e.g. cysteine or chelating agents like EDTA.

- High cell yields and viability
- Reliable lot-to-lot consistency
- TSE safe manufacturing
- Low endotoxin
- GMP Grade available



Myocytes, by courtesy of innovacell biotechnologie GmbH, Austria

Isolation procedures of defined cell types from different species require individual protocols.  
The tables below show digestion conditions using Collagenase NB qualities.

Cell type	Tissue	Species	Collagenase NB	Concentration	Experimental conditions		
Acinar cells	Pancreas	Human	NB 8	0.05 – 0.1 PZ U/ml	1 h, 37°C		
Adipocytes	Adipose	Human, mouse	NB 4 (NB 5 or NB 6), NB 4G	0.2 – 0.3 PZ U/ml *	30 – 45 min, 37°C		
Amnion cells	Amnion	Human	NB 8	3.5 – 5.5 PZ U/ml	3 – 5 h, 37°C		
Cardiomyocytes	Heart	Rat (adult)	NB 8	0.5 PZ U/ml	45 – 60 min (recirculation and digestion)	If required hyaluronidase, pancreatin or DNase I	
		Human	NB 8	0.8 – 1.0 PZ U/ml	2 – 3.5 h, 37°C		
Chondrocytes	Cartilage	Mouse (newborn)	NP** and NB 4 (NB 5 or NB 6)	1) NP: 0.5 – 1 DMC U/ml 2) NB 4: 0.3 PZ U/ml	1) NP: 15 – 20 min, 37°C 2) collagenase: 30 – 45 min, 37°C	Sequential digestion steps	
		Human	NB 4 (NB 5 or NB 6)	0.3 – 0.4 PZ U/ml	4 – 16 h, 37°C		If required Neutral Protease NB
Chondrocytes (articular)	Cartilage	Bovine	NB 4 (NB 5 or NB 6)	0.2 – 0.3 PZ U/ml	14 – 16 h, 37°C		
Chondrocytes (meniscal)	Fibrocartilage	Bovine	NB 4 (NB 5 or NB 6)	0.2 – 0.3 PZ U/ml	14 – 16 h, 37°C		
Chorion cells	Chorion	Human	NB 8	3.5 – 5.5 PZ U/ml	3 – 5 h, 37°C		
Chromaffin cells	Adrenal gland	Bovine, rat	NB 4 (NB 5 or NB 6)	0.3 – 0.5 PZ U/ml	20 – 30 min, 37°C		
Endometrial cells	Endometrium	Human	NB 4 (NB 5 or NB 6)	0.2 – 0.3 PZ U/ml	30 min, 37°C		
Endothelial cells (HDMEC)	Foreskin	Human	NB 4 (NB 5 or NB 6), NB 4G	0.5 – 0.7 PZ U/ml	0.5 – 2 h, 37°C		
Endothelial cells (HUVEC)	Umbilical cord	Human	NB 4 (NB 5 or NB 6)	0.15 – 0.25 PZ U/ml	0.5 – 2 h, 37°C	If required hyaluronidase	
Epithelial cells	Mammary gland	Mouse	NB 4 (NB 5 or NB 6)	0.34 PZ U/ml	15 – 25 min, RT		
	Thyroid glands	Human	NB 4G	0.25 – 0.3 PZ U/ml	RT		If required dispase
Fibroblasts	Skin	Human	NB 4G	0.26 PZ U/ml	2 h, 37°C		
	Skin, connective tissue	Rodent	NB 4G	0.2 – 0.5 PZ U/ml	12 – 20 h, 37°C		
Hepatocytes	Liver	Pig	NB 8	0.2 – 0.3 PZ U/ml	Perfusion and subsequent digestion		
		Mouse	NB 4G	0.08 – 0.11 PZ U/ml	5 – 15 min, 37°C (perfusion 5 – 8 ml/min)	Total collagenase perfusion volume 90 – 110 ml	
		Rat	NB 4G	0.12 – 0.15 PZ U/ml	10 – 25 min, 37°C (perfusion 5 – 8 ml/min)	Total collagenase perfusion volume 100 – 125 ml	
Hepatocytes/hepatic stellate cells	Liver	Human	NB 4G	0.25 – 0.35 PZ U/ml	10 – 20 min, 37°C		
Islets of Langerhans	Pancreas	Human	NB 1 and NP**	Per gram tissue: 20 PZ U (NB 1) and 0.8 – 1.5 DMC U (NP)	15 – 30 min, 37°C		
		Rodent	NB 8	1.5 – 1.7 PZ U/ml	14 – 17 min, 37°C (perfusion and subsequent digestion)		
		Pig (adult)	NB 1 and NP**	Per gram tissue: 15 – 18 PZ U (NB 1) and 0.8 – 1.3 DMC U (NP)	15 – 30 min, 37°C		
			NB 8 and NP**	Per gram tissue: 4.4 PZ U (NB 8) and 0.5 – 0.7 DMC U (NP)	37°C		
		Pig (neonatal)	NB 4 (NB 5 or NB 6)	0.17 – 0.2 PZ U/ml	10 – 20 min, 37°C		
Lung cells	Lung	Mouse	NB 4 (NB 5 or NB 6)	0.2 PZ U/ml	1 h, 37°C		
Mastocytes	Intestinal mucosa	Human	NB 4G	0.5 PZ U/ml	1 h, 37°C		
Melanoma cells	Skin	Mouse	NB 8	0.12 PZ U/ml	30 min, 37°C		
Muscle cells	Skeletal muscle	Human	NB 8	0.4 – 0.6 PZ U/ml			
Neurons	Dorsal root ganglia	Human, rodent	NB 4 (NB 5 or NB 6)	0.15 – 0.25 PZ U/ml	30 – 60 min, 37°C		
Oocytes	Ovary	Xenopus	NB 4G	0.3 – 0.5 PZ U/ml	1.5 – 2 h, RT		
Osteoblasts	Bone	Mouse	NB 4 (NB 5 or NB 6)	0.15 – 0.25 PZ U/ml	5 – 10 min, 37°C (4 – 5 repeats) and 0.2% dispase	Sequential digestion steps	
		Human	NB 8	1.5 – 2 PZ U/ml (NB 8) and 0.25 DMC U/ml (NP)	2 – 4 h, 37°C		
Schwann cells	Sciatic/peripheral nerve	Rodent	NB 4 (NB 5 or NB 6)	0.2 – 0.3 PZ U/ml	1 – 3 h, 37°C		If required trypsin or hyaluronidase
Stem cells	Wharton's jelly	Human	NB 4 (NB 5 or NB 6)	0.13 – 0.15 PZ U/ml	1 – 6 h, 37°C		If required hyaluronidase 1 mg/ml
Stem cells (ADSC)/preadipocytes	Lipoaspirate	Human, mouse	NB 4 (NB 5 or NB 6), NB 4G	0.2 – 0.3 PZ U/ml *	30 – 60 min, 37°C		
Tumor cells	Brest, uterus, thyroid gland, lung	Human	NB 8	3.5 – 5.5 PZ U/ml	3 – 5 h, 37°C		
	Colon		NB 4G	0.5 – 1 PZ U/ml	RT		

\* 0.1 – 0.15 PZ U/ml final collagenase concentration after addition of adipose tissue

\*\* Neutral Protease NB

Application	Cell type	Species	Collagenase NB	Concentration	Experimental conditions
Stem cell passaging	Human embryonic stem cells (hESC)	Human	NB 4 (NB 5 or NB 6)	0.15 PZ U/ml	37°C, 6 – 8 min

#### SERVA enzymes for research applications

**Collagenase NB 1 Premium Grade** is a highly purified collagenase with very low proteolytic side activities. For some applications, addition of purified **Neutral Protease NB** is required.

**Collagenase NB 4 Standard Grade** and **Collagenase NB 4G Proved Grade** are crude collagenases with a balanced mix of proteolytic activities.

**Collagenase NB 5 Sterile Grade** is characterized by comparable enzymatic properties to Collagenase NB 4 Standard Grade, but is manufactured under aseptic conditions (acc. Pharm. Eur.).

**Collagenase NB 8 Broad Range** is purified and contains higher collagenase and reduced proteolytic side activities.

#### GMP compliant enzymes for clinical requirements

Highly purified **Collagenase NB 1 GMP Grade** and **Neutral Protease NB GMP Grade** show enzymatic properties that are comparable to Collagenase NB 1 Premium Grade and Neutral Protease NB, respectively.

**Collagenase NB 6 GMP Grade** shows comparable enzymatic properties to Collagenase NB 4 Standard Grade but is particularly suitable for applications which have to meet cGMP requirements like tissue engineering, isolation and passaging of stem cells dedicated for transplantation into humans. Therefore after establishing isolation procedures with Collagenase NB 4 Standard Grade you may switch easily to Collagenase NB 6 GMP Grade.

#### Ordering Information

Collagenase	Abbr.	Cat. No.	Size
NB 1 Premium Grade	NB 1	17455.03	≥ 2000 U
NB 1 GMP Grade	NB 1 GMP	17452.01	≥ 2000 U
NB 4 Standard Grade	NB 4	17454.02	500 mg
		17454.01	1 g
NB 4G Proved Grade	NB 4G	17465.01	500 mg
		17465.02	1 g
NB 5 Sterile Grade	NB 5	17459.03	1 g
NB 6 GMP Grade	NB 6	17458.03	1 g
NB 8 Broad Range	NB 8	17456.01	250 mg
		17456.02	1 g

Enzyme	Abbr.	Cat. No.	Size
Neutral Protease NB	NP	30301.11	50 DMC U
		30301.12	100 DMC U
Neutral Protease NB GMP Grade	NP GMP	30303.01	≥ 100 DMC U

*SERVA Collagenase NB is not intended for use in humans. Responsibility for use of these enzymes in clinical applications and/or for isolation, purification and transplantation of cells lies solely with the operating physician/researcher.*

#### Related products

Product	Cat. No.	Size
Hyaluronidase	25118.01	50 mg
	25118.02	500 mg
DNase I	18535.01	25 mg
	18535.02	100 mg
Pronase E	33635.01	250 mg
	33635.02	1 g
	33635.03	5 g
Pancreatin NB	31442.01	100 g
	31442.02	250 g
Pancreatin NB GMP Grade	31445.01	250 g
Elastase from human neutrophils	20927.01	100 µg
Elastase from porcine pancreas, 120 U/mg	20931.01	25 mg
	20931.02	100 mg
Elastase from porcine pancreas, 200 U/mg	20929.01	10 mg
	20929.02	25 mg
Clostripain NB from <i>Clostridium histolyticum</i>	17335.01	20 mg
	17335.02	50 mg
	17335.03	100 mg
Trypsin 1:250 from bovine pancreas	37289.01	25 g
	37289.02	100 g

