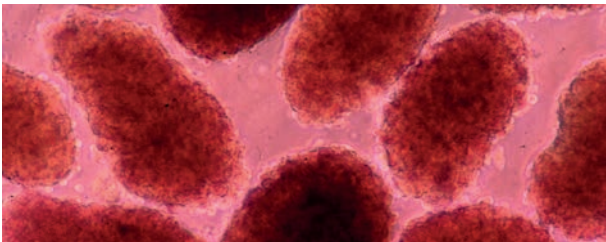


Neutral Protease NB Application Details for Tissue Dissociation

Collagenase NB from Nordmark

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 products 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 certificate of origin.



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

GMP Grade Enzymes

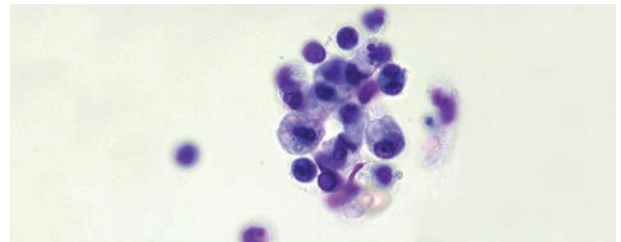
Enzymes for tissue dissociation are critical raw materials for tissue engineering and transplantation into humans. Thus, Nordmark Biochemicals offers Animal-free superior enzymes for the highest possible safety for clinical applications and superior GMP grade Collagenase NB and Neutral Protease NB products that are manufactured in compliance with the EU-Guide to Good Manufacturing Practice (GMP). Furthermore, TSE safety of the products is certified. Virus validation studies and, for each lot, testing for abnormal toxicity (according European Pharma-copoeia) 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 products 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 Wünsch and shows an optimum at 37 °C and at pH ~7.4. As collagenase requires calcium for full catalytic activity 2 mM Ca²⁺ is recommended.

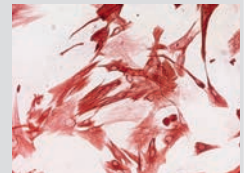


Human adipocytes, by courtesy of Pharmicell Europe GmbH, Germany

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 products.

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	Breast, uterus, thyroid gland, lung	Human	NB 8	3.5 – 5.5 PZ U/ml	3 – 5 h, 37 °C	
	Colon	Human	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

Technical Notes

Collagenase NB for Research Applications

Collagenase NB 1 Premium Grade is a highly purified collagenase with very low proteolytic side activities. For most 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. Ph. 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 GMP 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.

Collagenase AF-1 GMP Grade is completely free of any animal based components eliminating the potential for animal derived pathogens. Collagenase AF-1 GMP and Neutral Protease AF GMP are highly active and defined products designed to isolate sensitive cells from tissues such as liver, dental pulp or pancreas.

For information and samples please contact us:

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www.crestedchemical.com

Ordering Information

Enzyme	Abbr.	Cat. No.	Size
Collagenase NB 1 Premium Grade	NB 1	S1745503	≥ 2000 U
Collagenase NB 1 GMP Grade	NB 1 GMP	N0002937	≥ 2000 U
AF-1 GMP Grade	AF-1 GMP	N0003554	≥ 2000 U
Collagenase NB 4 Standard Grade	NB 4	S1745402	500 mg
		S1745401	1 g
Collagenase NB 4G Proved Grade	NB 4G	S1745403	5 g
		S1746501	500 mg
		S1746502	1 g
		S1746503	5 g
Collagenase NB 5 Sterile Grade	NB 5	N0002778	1 g
Collagenase NB 6 GMP Grade	NB 6	N0002880	100 mg
		N0002779	1 g
Collagenase NB 8 Broad Range	NB 8	S1745601	250 mg
		S1745602	1 g

Enzyme	Abbr.	Cat. No.	Size
Neutral Protease NB	NP	S3030111	50 DMC U
		S3030112	100 DMC U
Neutral Protease NB GMP Grade	NP GMP	N0002936	≥100 DMC U
Neutral Protease AF-1 GMP Grade	AF-1 GMP	N00033553	≥100 DMC U



Developed for *in vitro* tissue dissociation. Not suitable for direct use in humans!

Ordering

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