

Is There A Need For Recombinant Collagenase In Islet Isolation?

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Collagenase in Islet Isolation

- Which enzymatic characteristics are essential for a good isolation?

Dissociation efficacy of Collagenase Class I and Class II (rat pancreas):

II+I >> II > I > Neutral Protease >> no enzyme

(Vos-Scheperkeuter et al. (1997) Cell Transplant. **6**, 403-412)

Presence of 125 kD band in Collagenase P as indicator for efficient lot (pig)

(Chen et al. (2001) Cell Transplant. **10**, 709-716)

Ratio II/I = 0.7 to 1.0 optimal with respect to yield, viability, function (rat islets)

(Brandhorst et al. (2005) Transplant Proc. **37**(1), 215-6)

Dosage of Neutral Protease NB (human)

(Brandhorst et al. (2005) Transplant Proc. **37**(1), 241-2)



Collagenase in Islet Isolation

- **Recombinant collagenase (Class I + Class II)**

Recombinant class I combined with “native” class II result in similar yield and digestion time as native class I and II

(Wolters et al. (1995) Diabetes **44**, 227-233)

Isolation results with respect to yield, viability and function comparable to Liberase HI

(Brandhorst et al. (2003) Diabetes 52, 1143-1146)

- **Additional protease is essential**

Neutral Protease (*Clostridium histolyticum*)

Thermolysin (*Bacillus thermoproteolyticus*)

Dispase (*Bacillus polymyxa*)

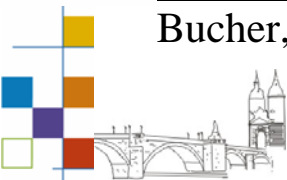


Islet Isolation Results with Collagenase NB 1 and Neutral Protease NB

- **Human Islet Isolation Results:**
Comparison Collagenase NB 1 / Neutral Protease NB and Liberase

	Collagenase NB 1 / Neutral Protease NB	Liberase	P value
IEQ (10^3)	354.4 ± 65	271.9 ± 147	0.08
IEQ/g pancreas (10^3)	4.02 ± 1.20	2.36 ± 1.35	<0.05
IEQ/Islet number ratio	0.83 ± 0.2	0.51 ± 0.1	<0.01
Purity (%)	62 ± 6	53 ± 8	<0.05
Tissue volume (ml)	3.8 ± 1.6	4.2 ± 2.1	0.3
Islet Viability (%)	96 ± 1	94 ± 2	0.1
Stimulation Index	4.3 ± 1.5	3.8 ± 3.2	0.3
Apoptosis after 12h culture	1.25 ± 0.03	7.25 ± 1.23	<0.05

Bucher, P., Berney, T. et al. (2005) Transplantation 79(1), 91-97.



Collagenase in Islet Isolation

- **In principle, recombinant and non-recombinant collagenase/protease give good isolation results**

Problems: Lot-to-lot consistency and stability have to be assured



Question: How can these problems be solved?



Collagenase in Islet Isolation

➤ Different approaches:

- **Recombinant enzyme production**
- **Non-recombinant enzyme production according to GMP**

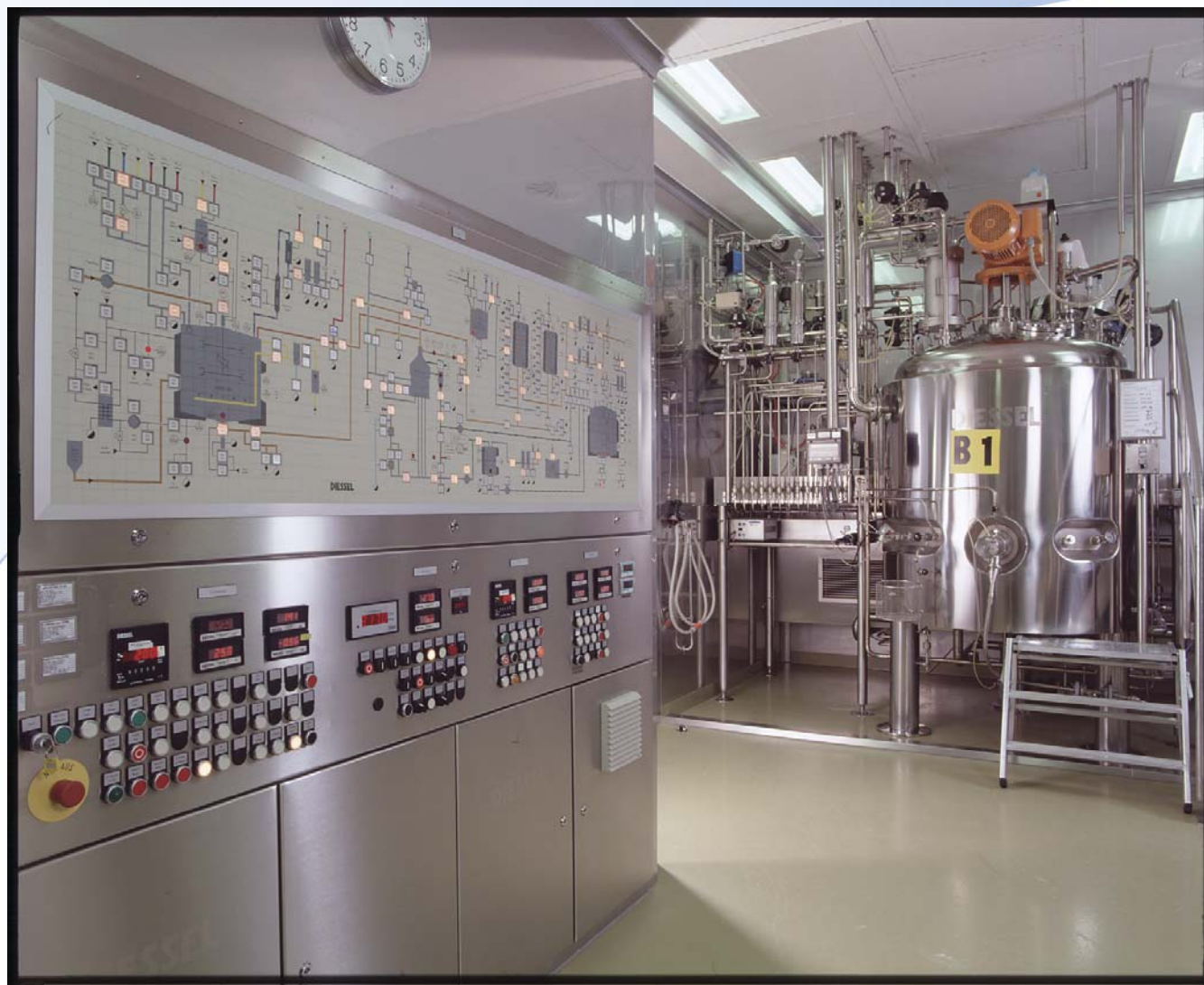


GMP compliance / documentation

- **Production compliant to international GMP guidelines**
 - Highly reproducible batches
 - Validation of production process
 - Complete production documentation available (Traceability)
 - Dedicated production facility (no cross contamination possible)
 - Qualified personnel
 - Validated and calibrated equipment
 - Stability Study (compliant to ICH guidelines Q1A for drug substances)
- **Safety issues**
 - Test for Abnormal Toxicity according to Pharm. Eur.
 - Virus validation studies
 - Certificate of Suitability (CoS-TSE) from European Authorities EDQM for fermentation ingredients
- **Filing of Drug Master File (US) , PTA (FR)**



Production of Collagenase N: Fermentation

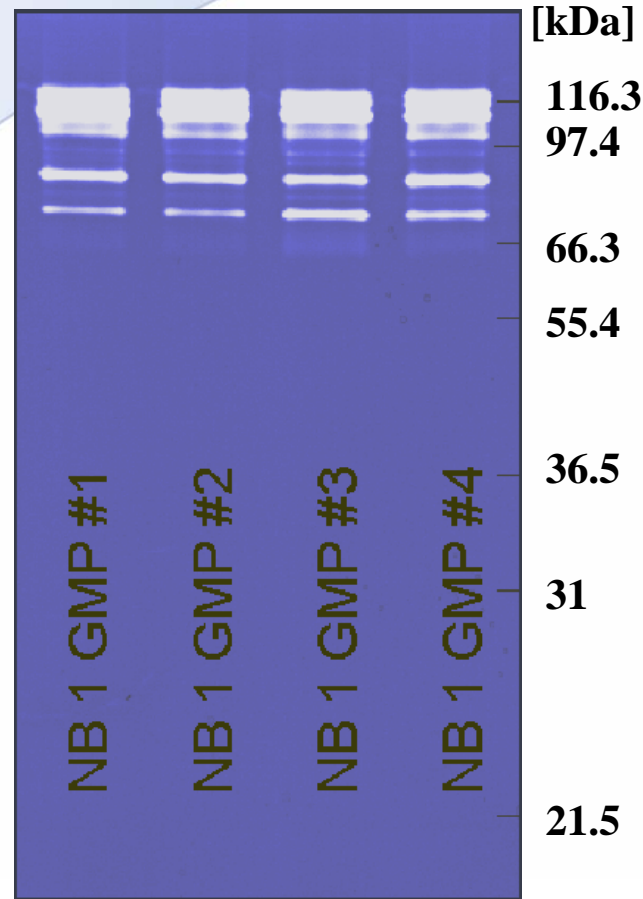


Production of Collagenase NB: Chromatography System



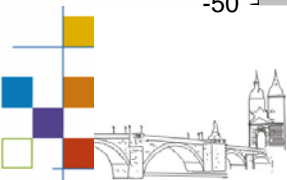
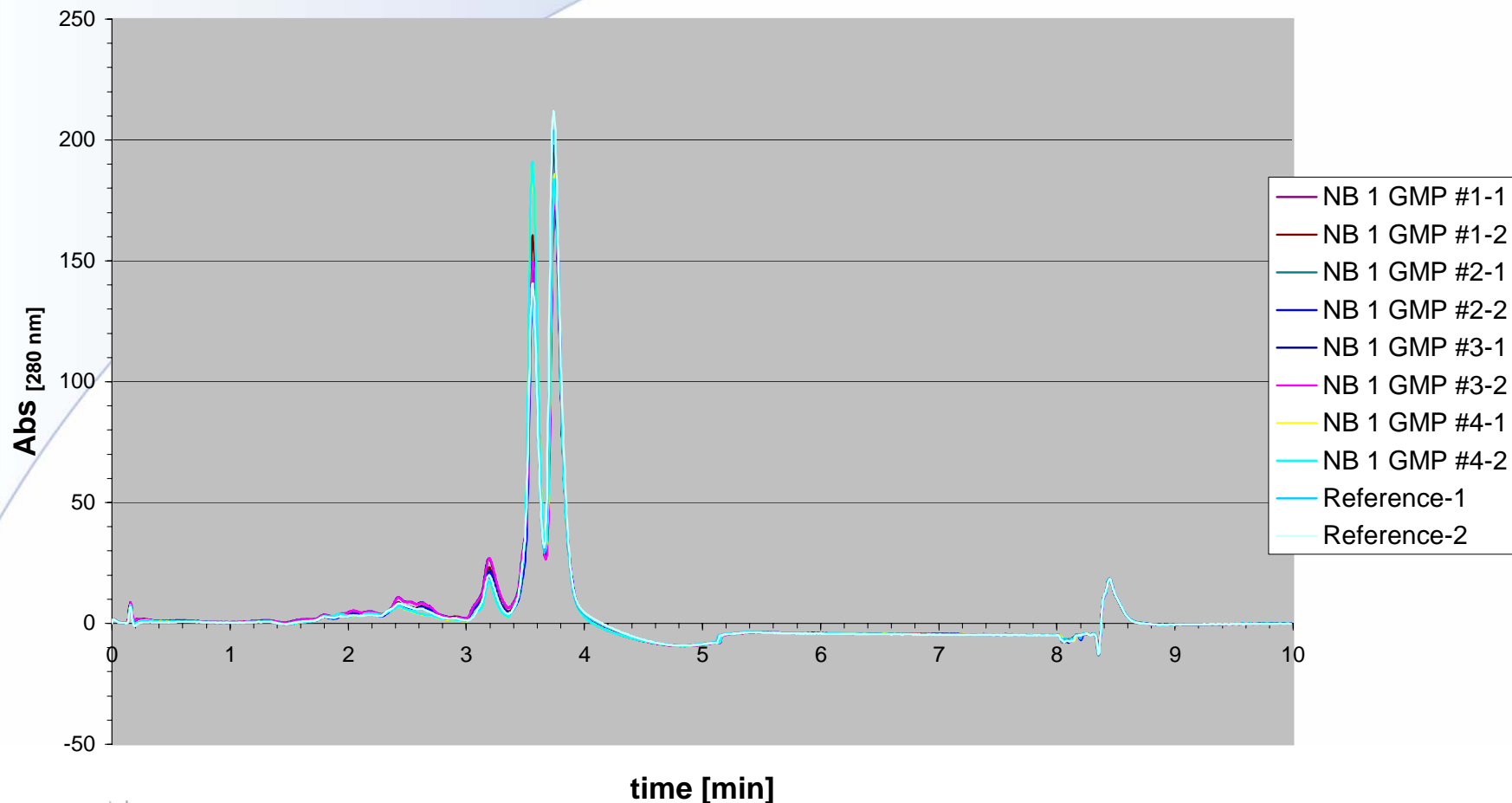
Lot-to-lot consistency: Collagenase NB 1 GMP Grade

Gelatin Zymogram of Collagenase NB 1 GMP Grade



Lot-to-lot consistency: Collagenase NB 1 GMP Grade

RP-HPLC of Collagenase NB 1 GMP Grade



Stability Data of Collagenase NB 1 GMP Grade and Neutral Protease NB GMP Grade

- **Stability study according to ICH guidelines (Int. Conference on Harmonisation)**

Preliminary results after 18 months for lyophilized enzymes in glass vials:

	Storage temperature	Storage duration
Collagenase NB 1	+5 °C	18 months
Neutral Protease NB	-25 °C and -85 °C	18 months
	+5 °C	6 months

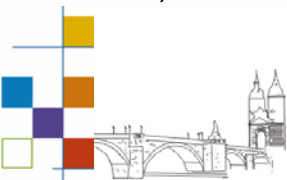
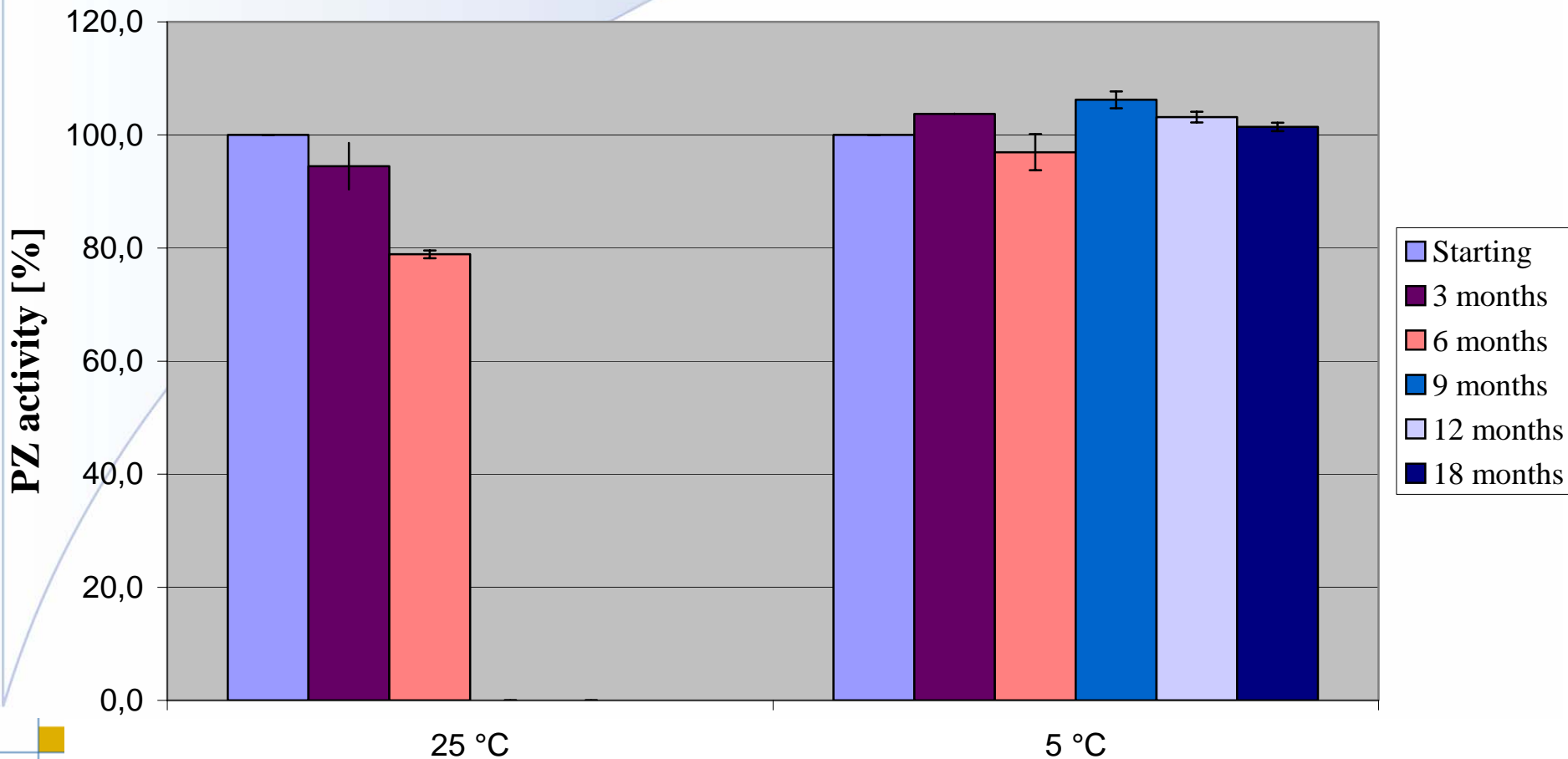
Final results after 12 months for solubilized enzymes in PE cups:

	Storage temperature	Storage duration
Collagenase NB 1	-25 °C and -85 °C	12 months
	50 Pz U/mL in Tris	
Neutral Protease NB	-25 °C and -85 °C	12 months
	10 mg/mL in water	



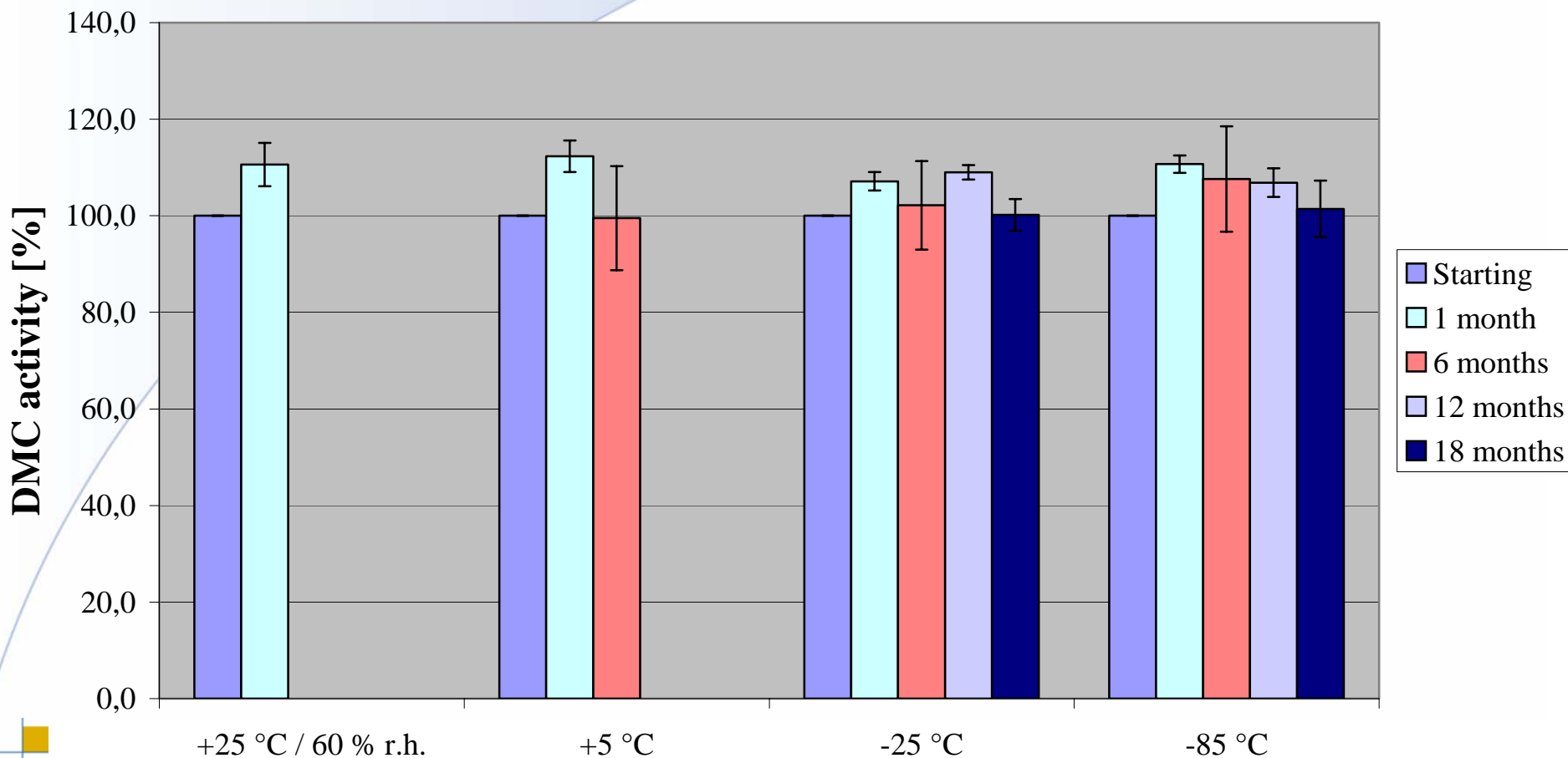
Stability Data: Collagenase NB 1 GMP Grade

Stability of Collagenase NB 1 GMP Grade (Lyophilisate)



Stability Data: Neutral Protease NB GMP Grade

Stability of Neutral Protease NB GMP Grade (Lyophilisate)



Summary

- **Collagenase NB 1/Neutral Protease NB, Liberase HI and recombinant collagenase show comparable efficacy in islet isolation**
- **Recombinant enzyme production for islet isolation:**

Three enzymes have to be produced separately (collagenase class I, collagenase class II, neutral protease) in complex expression systems.

➔ Lot-to-lot consistency is difficult to achieve
- **GMP compliant production only for Collagenase NB 1/Neutral Protease NB:**
 - Highly reproducible lots
 - Stability data available
 - Safe product
 - Fulfills all requirements of the authorities

