Protocol for rat cardiomyocyte isolation

Product Information

General	Collagenase NB 8 is used for dissociation of tissues of different species (e.g. human, rat, pig) for isolation of various cell types. The Collagenase NB 8 producing strain of *Clostridium histolyticum* has been carefully selected for producing high amounts of collagenase and a balanced ratio of secondary proteases. Collagenase NB 8 is chromatographically purified and therefore contains reduced levels of secondary proteases and endotoxin.
Specification	Collagenase activity ≥ 0.9 U/mg (PZ acc. to Wünsch) Neutral protease activity ≤ 0.2 U/mg (DMC) Trypsin-like activities ≤ 0.5 U/mg (BAEE) Endotoxin ≤ 100 EU/mg
Application	Collagenase NB 8 is used for dissociation of tissues of different species (e.g. human, rat, pig) for isolation of various cell types which are used for research purposes. The enzyme is not intended for use in humans.
Storage conditions	+2 to +8 °C

Instruction for use

General	Below, a protocol is described to achieve optimal isolation results for rat cardiomyocytes with Collagenase NB 8.
Chemicals/ Solutions	• Krebs Henseleit buffer (without Ca^{2+}): 118 mM NaCl (6.9 g/L) 4.7 mM KCl (0.35 g/L) 1.64 mM $MgCl_2$ (0.14 g/L) 24.88 mM $NaHCO_3$ (2.09 g/L) 1.18 mM KH_2PO_4 (0.16 g/L) 5.55 mM Glucose (1.09 g/L) 2.0 mM Na-Pyruvat (0.22 g/L) CO_2 gas saturation • Collagenase NB 8, SERVA Electrophoresis (Cat. No. 17456) • Hyaluronidase (Sigma)
Buffers	**1. Perfusion Solution** • Krebs-Henseleit buffer (without Ca^{2+}, CO_2 saturated) **2. Collagenase/Hyaluronidase solution** • Krebs-Henseleit buffer (without Ca^{2+}, CO_2 saturated) + 0.5 mg/mL Collagenase NB 8 + 1 mg/mL Hyaluronidase The solution is kept cold until use.
Tissue perfusion	1. Coronary arteries are cleaned by retrograde perfusion through the aorta with perfusion solution. After 5 min, residual blood should be removed. 2. Collagenase/Hyaluronidase solution is warmed up to 37 °C and is applied. The heart is perfused with this solution for about 30 min until the organ gets “soft” and its contour is fainting.
Tissue dissociation	The heart is transferred into a petri dish with the enzyme solution. Dissociation is allowed to proceed under soft agitation at 37 °C.
Results	Yield: 6 to 8 million cells, up to 80 % intact (rod shaped). β-receptors, K_{ATP} channels, Ca^{2+} channels are intact.

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