

The Worthington Neonatal Cardiomyocyte Isolation System has been introduced to provide researchers with a reliable, convenient, and consistent cell isolation system. By utilizing purified rather than crude enzyme preparations, it has been possible to minimize the lot-to-lot variation. In addition, Worthington use-tests the kits by isolating cardiomyocytes from neonatal rat hearts to assure performance, reliability and consistent yield of viable cells.

The kit has been formulated in conjunction with Dr. Ronal MacGregor. The method is based on that described by Toraason, et al. (1988) in which the minced tissue is incubated overnight with purified trypsin at 4°C. As pointed out by Toraason, this step reduces the hands-on time required to harvest cells compared to the time involved in sequential incubations in warm trypsin or collagenase. Also, purified collagenase is utilized to maximize yield and viability. The kit includes five single-use vials each of purified trypsin, collagenase and trypsin inhibitor supplied along with Hank's Balanced Salt Solution, Leibovitz L-15 culture media, Falcon Cell Strainers and detailed instructions.

Description	Code	Size	Cat. No.
Neonatal Cardiomyocyte Isolation System	NCIS	1 Kit	LK003300
		3 Kits	LK003303
Individual Components			
Trypsin Vials	TRLSNK	1 vi	LK003220
		5 vi	LK003225
Collagenase Vials	CLSPANK	1 vi	LK003240
		5 vi	LK003245
Inhibitor Vials	SICNK	1 vi	LK003230
		5 vi	LK003235
Hank's Buffer	HBSS	500 ml	LK003210
L-15 Media Powder	L15NK	1 x 1L	LK003250
Cell Strainers	CELSTRNK	5 ea	LK003265

Description and Package Contents

The package contains sufficient materials for five separate tissue dissociations, each containing up to twelve hearts. For larger or smaller tissue samples prepare proportionate volumes of reagents at each step and combine them in the same ratio as described in the protocol.

(Over) [10.13]



Vial #1: HBSS, 1 bottle, 500 ml Sterile calcium and magnesium-free Hank's Balanced Salt Solution (CMF HBSS), pH 7.4. The solution is used for reconstituting the contents of Vials #2 and #3 in addition to serving as the medium for the dissociation.

Vial #2: Trypsin Vial, 5 vials, 1,000 µg each Worthington Trypsin (Code: TRLS), 3X crystallized, dialyzed against 1mM HCl, filtered through 0.22µm pore size membrane, and lyophilized. Before use, reconstitute with 2ml CMF HBSS (Vial #1) and swirl gently to dissolve contents. Store at 2–8°C.

Vial #3: Inhibitor Vial, 5 vials, 2,000 µg each Worthington Soybean Trypsin Inhibitor (Code: SIC), a 0.22µm pore size membrane filtered, lyophilized powder. This material is 0.22 micron membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 1 ml of HBSS or equivalent yields a solution of 2 mg/ml of trypsin inhibitor, Code SIC. Before use, reconstitute with 1ml CMF HBSS (Vial #1) and swirl gently to dissolve contents. Store at 2–8°C.

Vial #4: Collagenase Vial, 5 vials, 1,500 Units each Worthington Purified Collagenase (Code: CLSPA), a 0.22µm pore size membrane filtered, lyophilized powder which has been chromatographically purified. It contains less than 50 caseinase units per milligram and is composed of two separable but very similar collagenases. Before use, reconstitute with 5ml Leibovitz L-15 Media (prepared as described below) and swirl gently to dissolve contents. Store at 2–8°C.

Pouch containing Leibovitz L-15 Media Powder: 1x1 L Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing 800ml of cell culture grade water. Rinse pouch 2 - 3 times with additional 100ml. Bring total volume to 1 liter and filter through a 0.22µm pore size filter. Store at 2-8°C.

The kit also includes 5 Cell Strainers (Falcon), and a card correlating phenol red color with pH for checking the pH of balanced salt solution and culture medium.

References

MacGregor, Ronal R.: Hormone Regulated Secretion of Plasminogen Activator Activity from Neonatal Rat Cardiomyocytes, in publication.

Toraason, Mark, Luken, Mary E., Breitenstein, Michael, Krueger, John A., and Biagini, Raymond E.: Comparative Toxicity of Allylamine and Acrolein in Cultured Myocytes and Fibroblasts from Neonatal Rat Heart, *Toxicology*, 56, 107 1988.

Related Products

Cell Isolation Optimizing System
Collagenase
Elastase
Hepatocyte Isolation Kit
Hyaluronidase
Neutral Protease (Dispase®)
Papain
Papain (Neural) Dissociation System
STEMxyme™ 1 & 2 Collagenase/Neutral Protease Blends
Trypsin

**Complete Catalog, Tissue Dissociation Guide
and Enzyme Manual available online at:**

**Worthington-Biochem.com
TissueDissociation.com**