INSTRUCTIONS

Pierce Primary Cardiomyocyte Isolation Kit

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88281

Number	Description
88281	**Pierce Primary Cardiomyocyte Isolation Kit,** contains sufficient reagents to isolate cardiomyocytes from 50 neonatal mouse/rat hearts. Also contains reagents that support the culture of cardiomyocytes.

Kit Contents:

Cardiomyocyte Culture Module (88281X), store at 4°C:

DMEM for Primary Cell Isolation, 500mL

Hanks' Balanced Salt Solution (HBSS without Ca^{2+}/Mg^{2+}), 500mL

Cardiomyocyte Isolation Module (88281Y), store at -20°C: **Cardiomyocyte Isolation Enzyme 1 (with papain),** lyophilized, 5 vials **Cardiomyocyte Isolation Enzyme 2 (with thermolysin) (20X),** 100µL, 5 vials **Cardiomyocyte Growth Supplement (1000X),** 0.5mL

Storage: Upon receipt store Product 88281X at 4°C and Product 88281Y at -20°C. Product 88281X is shipped on ice packs and Product 88281Y is shipped with dry ice.

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Introduction

The Thermo Scientific™ Pierce™ Primary Cardiomyocyte Isolation Kit provides a validated method for the isolation and culture of primary cardiomyocytes from neonatal mouse/rat hearts. The kit consists of unique tissue-specific dissociation reagents and an optimized protocol to ensure a high yield of viable and fully functional cardiomyocytes when used by both experienced and non-experienced users. The entire isolation procedure, from processing primary tissues to seeding cells in culture vessels, can be completed within two hours. The fully optimized culture reagents are designed to provide optimal growth conditions for maintaining highly pure primary cardiomyocytes in culture.

Primary cardiomyocytes isolated and cultured using the Pierce Primary Cardiomyocyte Isolation Kit express cardiomyocyte protein markers and maintain contractile function. The fully functional cardiomyocytes may serve as a model system for a broad spectrum of experiments including contraction, ischaemia and hypoxia studies.[1] In combination with

immunofluorescent technologies, primary cardiomyocytes isolated and cultured with the Pierce Primary Cardiomyocyte Isolation Kit are well-suited for experiments aimed at visualizing cellular structure and molecular localization.

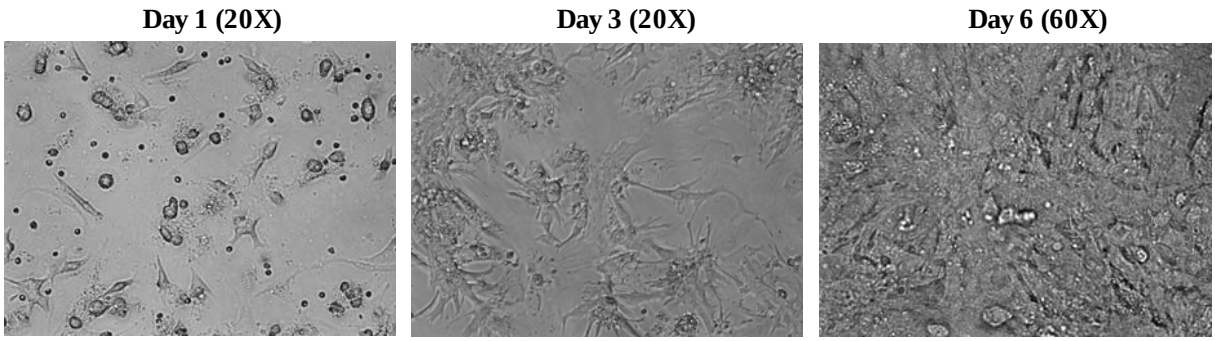

Figure 1. Developmental stages of cultured primary cardiomyocytes. Phase-contrast images of cultured neonatal mouse cardiomyocytes at 1, 3 and 6 days in culture. Cultures were plated in a 24-well plate at a density of 5 x 10^5 cells per well. Cell contractions were observed visually under a light microscope after 1 day in culture. The strong synchronous contractions of cells in the wells could be visualized by phase contrast microscopy after 3 days in culture. These cells beat spontaneously at an average rate of 115-145 beats/minute. Images were taken at 20X and 60X magnification as indicated.

Important Product Information

- For best cell yield and viability, always isolate cardiomyocytes from freshly dissected tissues. The dissection and plating of cardiomyocytes should take no more than two hours.
- Use Day 1-3 neonatal mouse/rat hearts to isolate cardiomyocytes.
- Euthanize mice or rats in accordance with the Guidelines for the Care and Use of Laboratory Animals.[2]
- Perform all tissue digestion and cell manipulations using sterile technique in a laminar flow cell culture hood to minimize contamination of the isolated cardiomyocytes.

Additional Materials Required

- Culture slides/dishes
- Heat-inactivated fetal bovine serum (FBS) (e.g., Thermo Scientific™ Hyclone™ FBS)
- Penicillin-streptomycin (pen/strep) (e.g., Thermo Scientific™ Hyclone™ Pen/Strep Solution)
- Sterile 1.5mL microcentrifuge tubes
- Day 1-3 neonatal hearts freshly dissected from mouse/rat
- 37°C heat block or incubator
- Tissue culture incubator at 37°C with humidified, 5% CO_2 atmosphere
- Laminar flow cell culture hood
- Hemocytometer or automated cell counter
- Trypan blue stain (e.g., Thermo Scientific™ Hyclone™ Trypan Blue)

Material Preparation

Note: After supplementation, medium is stable for approximately one month when stored at 4°C.

Complete DMEM for Primary Cell Isolation	Determine the amount of medium needed (see Table 2, pg. 4 for guidelines). In a sterile bottle, add heat-inactivated FBS (10% final concentration) and pen/strep (1% final concentration) to desired volume of DMEM for Primary Cell Isolation. Pre-warm medium to 37°C before use.

Procedure for Cardiomyocyte Isolation

A. Enzyme Digestion of Neonatal Heart Tissue

Note: For the medium and buffer removal steps, it is critical to carefully remove the medium/buffer contents without disturbing the cells. For best results, use a pipette and 1000µL tip. Do not aspirate using a vacuum flask.

Note: Chill HBSS to 4°C before procedure.

1. Reconstitute the Cardiomyocyte Isolation Enzyme 1 (with papain) by adding 2.4mL of HBSS to one of the vials. Mix gently for 5 minutes or until completely dissolved. Keep enzyme solution on ice.

   Note: 2.4mL reconstituted Cardiomyocyte Isolation Enzyme 1 (with papain) is sufficient for preparing 10 neonatal hearts.

   Note: Reconstituted Cardiomyocyte Isolation Enzyme 1 (with papain) can be stored at -20°C for 6 months and is stable for up to two freeze-thaw cycles. This enzyme solution expires one week following preparation if stored at 4°C. Enzyme use after prolonged storage may result in poor performance.

2. Place freshly dissected neonatal hearts into separate 1.5mL sterile microcentrifuge tubes. Immediately add 500µL ice cold HBSS.

   Note: For the best results, use one microcentrifuge tube per one neonatal heart.

3. Mince each heart into 1-3mm^3 pieces. Wash the minced tissue twice with 500µL ice cold HBSS to remove blood from the tissue.

4. Add 0.2mL reconstituted Cardiomyocyte Isolation Enzyme 1 (with papain) and 10µL Cardiomyocyte Isolation Enzyme 2 (with thermolysin) to each tube. Mix gently and incubate tubes in a 37°C incubator for 30-35 minutes.

   Note: Cardiomyocyte Isolation Enzyme 2 (with thermolysin) is supplied as a suspension in HBSS. Thaw on ice before use. Once thawed, ensure that the enzyme is in a uniform suspension by pipetting up and down several times before use.

5. Gently remove the enzyme solution and wash tissue twice with 500µL ice cold HBSS.

6. Add 0.5mL Complete DMEM for Primary Cell Isolation to each tube. Break up the tissue by pipetting up and down 25-30 times using a sterile 1.0mL pipette tip. Avoid air bubbles when pipetting.

   Note: Disrupting the tissue by pipetting improves cell yield. However, pipetting too vigorously can result in cell damage.

7. After the tissue is primarily a single-cell suspension, add 1.0mL Complete DMEM for Primary Cell Isolation to each tube to bring the total volume to 1.5mL.

8. Combine individual cell suspensions for determination of cell concentration and cell viability.

B. Cell Yield and Viability Determination

1. Mix 25µL single-cell suspension obtained in Section A, Step 8, with 25µL 0.4% trypan blue in a 1.5mL microcentrifuge tube.

2. Immediately transfer 10µL trypan blue-stained cell suspension to each of two hemocytometer counting chambers.

3. Count both the total number of cells and the number of stained (blue) cells from the hemocytometer microscopic grid.

   Cell concentration (cells/mL) = number of cells × dilution factor × 10^4

   Example: If 120 cells in a square, then $120 \times 2 \times 10^4 = 2.4 \times 10^6$ cells/mL

   Cell yield = cell concentration × volume of cell suspension, obtained in Section A, Step 8

   Viability (%) = [(total cells counted – total stained cells) / total cells counted] × 100%

   Typical cell yields and viabilities obtained are shown in Table 1.

4. If using an automated cell counter, determine cell yield and viability according to the manufacturer's instructions.

Table 1. Cell yield and viability from a typical isolation.

Cell Type	Yield (cells/mL)	Viability (Trypan Blue Exclusion)
Mouse cardiomyocytes (one neonatal heart in 1.5mL cell suspension)	2.0×10^6	63%
Rat cardiomyocytes (one neonatal heart in 1.5mL cell suspension)	2.5×10^6	62%

C. Plating and Culturing Isolated Cardiomyocytes

Note: Determine the desired plating density for the cultured cardiomyocytes based on the intended downstream study. In general, a seeding density of 2.5×10^5 cells/cm^2 is recommended for culturing cardiomyocytes.

1. Pipette the appropriate cell suspension volume into each well of the culture vessel (see Table 2, below):

   Cell suspension volume/well = [required cell density × growth area (cm^2)]/cell concentration (cells/mL from Section B, Step 3, above)

   Example: For a 24-well Nunc™ plate, a single well is approximately 1.8cm.^{2} If the cell concentration is 2×10^6 cells/mL, add 225µL of cell suspension to each well.

Table 2. Recommended seeding densities for common culture vessels.*

Nunc Culture Dishes/ Chamber Slides	Well Diameter (mm)	Approximate Growth Area (cm^2)	Medium Volume (mL)	Total Number of Cells Required to Seed Each Well
35mm dish	35	9.0	2.0	2.3×10^6
6-well plate	35	9.6	2.0	2.5×10^6
12-well plate	22	3.5	0.8	1.0×10^6
24-well plate	16	1.8	0.5	5.0×10^5
48-well plate	11	1.1	0.3	3.0×10^5
96-well plate	4.3	0.14	0.1	4.0×10^4
4-well chamber slide	NA	1.8	0.5	5.0×10^5
8-well chamber slide	NA	0.8	0.2	2.0×10^5

* Additional medium may be required for long-term cultures.

2. Add Complete DMEM for Primary Cell Isolation to each well to bring the total volume to the recommended level.

   Example: For the 24-well Nunc plate example in Step 1, add 275µL Complete DMEM for Primary Cell Isolation to each well to bring the total volume to 0.5mL.

3. Incubate the dishes/chamber slides at 37°C in a 5% CO_2 incubator for 24 hours.

4. After 24 hours, replace the medium with an equivalent volume of fresh Complete DMEM for Primary Cell Isolation containing Cardiomyocyte Growth Supplement diluted 1000-fold.

Example: For a 24-well Nunc plate, prepare a total volume of 12mL Complete DMEM for Primary Cell Isolation. Add 12µL Cardiomyocyte Growth Supplement to the medium and mix. For small volumes, dilute the Cardiomyocyte Growth Supplement ten-fold in HBSS before use to avoid pipetting errors. Do not store diluted Cardiomyocyte Growth Supplement.

Note: Cardiomyocyte Growth Supplement reduces fibroblast contamination and maintains cardiomyocytes at a high purity during the culture period. Cardiomyocyte purity in culture at Day 7 is expected to be over 80% when using the recommended dilution of Cardiomyocyte Growth Supplement.

5. Incubate the cultures at 37°C in a 5% CO_2 incubator.

   Note: After the first medium change, subsequent medium changes can be carried out every 3 days following the procedure in Step 4, above.

Troubleshooting

Problem	Possible Cause	Solution
Low yield/low viability	Insufficient dissociation	Use freshly reconstituted Cardiomyocyte Isolation Enzyme 1 (with papain)
		Confirm Cardiomyocyte Isolation Enzyme 1 (with papain) and Cardiomyocyte Isolation Enzyme 2 (with thermolysin) concentrations. Use the recommended amount
		Follow recommended incubation time
		Check expiration date on the product. Do not use after expiration date
		Pipette tissue to aid in dissociation of cardiomyocytes
	Over-digestion	Do not exceed the recommended incubation time of 40 minutes in Section A, Step 4
		Confirm that Cardiomyocyte Isolation Enzyme 1 (with papain) is reconstituted to the recommended concentration
	Cells are dead or damaged	Do not over-pipette the cardiomyocytes during dissociation
Slow cell growth	Medium and/or supplement stored incorrectly, or expired medium and/or supplement	Check the recommended storage conditions on pg. 1. Confirm that the product components were stored properly
		Do not use expired medium and/or supplements
Low cell purity	Did not use Cardiomyocyte Growth Supplement	Add Cardiomyocyte Growth Supplement at the recommended concentration at Day 1 after seeding cells and for all subsequent medium changes

Related Thermo Scientific Products

88287	**DMEM for Primary Cell Isolation**
88288	**Cardiomyocyte Isolation Enzyme 1 (with papain)**
88289	**Cardiomyocyte Isolation Enzyme 2 (with thermolysin)**
88284	**Hanks' Balanced Salt Solution (HBSS, without Ca^{2+}/Mg^{2+}),** 500mL
88280	**Pierce Primary Neuron Isolation Kit**
88285	**Neuronal Isolation Enzyme (with papain)**
88286	**Neuronal Culture Media Supplement**
88283	**Neuronal Culture Medium,** 500mL
88279	**Pierce Mouse Embryonic Fibroblast Isolation Kit**
88290	**Mouse Embryonic Fibroblast Isolation Enzyme (with papain)**
87790	**Subcellular Protein Fractionation Kit for Tissue**
78510	**T-PER™ Tissue Protein Extraction Reagent**

References

1. Chlopcikova, S., *et al*. (2001). Neonatal Rat Cardiomyocytes – A Model for the Study of Morphological, Biochemical and Electrophysiological Characteristics of the Heart. *Biomed. Papers* **145(2):**49-55.
2. National Research Council of the National Academies (2011). The Guide for the Care and Use of Laboratory Animals. *The National Academies Press*. Eighth Edition.