Cardiosight-S®

user guide

WE MAKE HUMAN CELLS

Contents

User Notice & Restrictions:

1. User may use the Product for internal research including but not limited to screening potential drug compounds for efficacy and safety, and for the provision of such services to third parties.
   No other right is granted to User whether expressly, by implication, by estoppel or otherwise.
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2. User agrees to use the Product in compliance with all applicable statutes and regulations, but not to use the Product for any administration or application to humans.
   Moreover, User agrees not to use the Product in human subjects for human clinical use for therapeutic, diagnostic or prophylactic purposes, or in animals for veterinary use for therapeutic, diagnostic or prophylactic purposes, including but not limited to clinical applications, cell therapy, transplantation, and/or regenerative medicine without an appropriate license.

3. In the case that User transfers Product to a third party, User shall convey the User Restrictions set forth herein to such third party.

NEXEL

Prediction of cardiotoxicity for drugs using

human induced pluripotent stem cell-derived cardiomyocytes

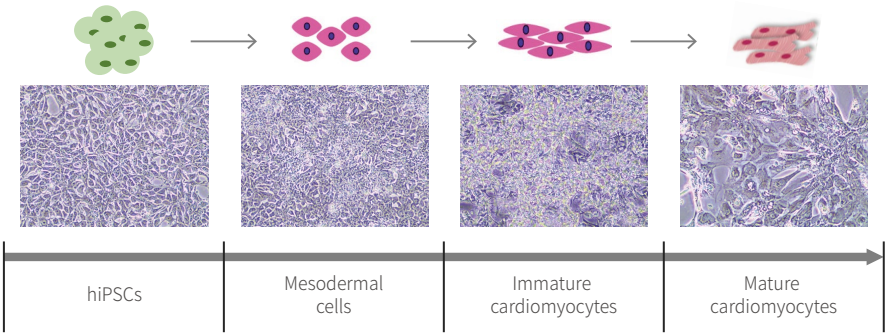
1. Introduction

Cardiosight-S® is a highly pure population of cardiomyocytes differentiated from human induced pluripotent stem cells (hiPSCs).

2. Characteristics of Cardiosight-S®

2-1. Morphology

The Cardiosight-S® was induced using NEXEL's proprietary protocol *in vitro*.

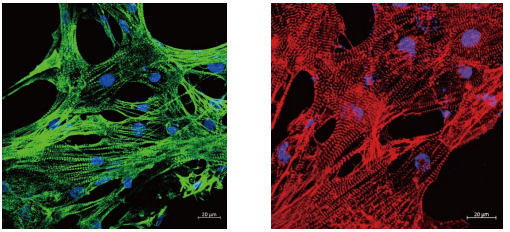


Summary of Cardiosight-S® induction process and representative images of cell morphology at each step.

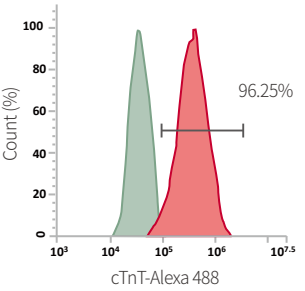
2-2. Cytochemical Analysis

Cardiosight-S® expresses cardiomyocyte markers (cTnT, α-actinin) and is a highly pure population of Cardiomyocyte (cTnT ≥ 95%).

Cardiomyocyte marker expression



Pure Cardiomyocytes



3. List of Components (provided by NEXEL)

Cardiosight-S® (C-001)

Item	Cat. #	Storage	Note
Cardiosight-S®	C-001	≤-150°C	hiPSC-derived Cardiomyocytes, ≥2 X 10^6cells
Cardiosight-S® Media	CM-001	4°C	50 ml
Cardiosight-S® Supplement (100X)	CS-001	-20°C	500 µl

Cardiosight-S® (C-002)

Item	Cat. #	Storage	Note
Cardiosight-S®	C-002	≤-150°C	hiPSC-derived Cardiomyocytes, ≥5 X 10^6cells
Cardiosight-S® Media	CM-002	4°C	100 ml
Cardiosight-S® Supplement (100X)	CS-002	-20°C	1 ml

4. Additional Materials Required (Not provided by NEXEL)

Item	Vendor	Cat. #
DMEM/F12	Multiple Vendors	-
Matrigel	Corning	354277
Y-27632	Tocris	1254
Fibronectin	Sigma	F1141

These specific items are highly recommended to be used for most optimal result.

5. Storage

- Cardiosight-S® should be stored at ≤–150°C (Liquid Nitrogen tank).
- Cardiosight-S® Media should be stored at 4°C and used before the expiry date indicated on the label.
- Cardiosight-S® Supplement should be stored at -20°C.

6. Preparing cell culture

6-1. Media

3 different types of Media are needed to generate mature cardiomyocytes and the Media can be prepared by mixing the following components :

Type of Media	Components
Thawing Media	Cardiosight-S® Media
Plating Media	Cardiosight-S® Media + Y27632 10 μM
Maintaining Media	Cardiosight-S® Media + Cardiosight-S® Supplement (100X)

Note: For the Maintaining Media, use 1X of the Cardiosight-S® supplement.

6-2. Plate (coating matrix)

1. Prepare the coating solution by adding Matrigel (10 μl/ml) in DMEM/F12.
2. Select the cell culture plate appropriate for your use.
3. Add the recommended amount of diluted-matrigel solution to each well of the cell culture plate.

Cell culture plate	6 well	12 well	24 well	48 well	96 well
Recommended amount of diluted - matrigel	1000 μl	500 μl	300 μl	100 μl	50 μl

Note: Use fibronectin (50ug/ml) for coating multi electrode plate.

4. Gently swirl the plate to distribute liquid evenly.
5. Incubate the plate at room temperature for 1 hour.
6. Replace the Matrigel solution with the Plating Media before plating the cell (section8).

7. Thawing procedure

1. Equilibrate the Thawing Media and Plating Media in a 37°C water bath before thawing Cardiosight-S®.
2. Remove the Cardiosight-S® cryovial from the liquid nitrogen storage tank.
3. Put the cryovial in a 37°C water bath for exactly 1 minute.
4. Remove the cryovial from the water bath, spray with 70% Et-OH, and then place it in a safety hood.
5. Gently add 1ml of Thawing Media into cryovial.
6. Carefully transfer the thawed cells into a conical tube which contains 9ml of Thawing Media.
7. Gently mix the cell suspension by slowly inverting.
8. Centrifuge the suspended cells at 1200rpm for 3 minutes at room temperature.
9. Carefully discard the supernatant.
10. Add 1ml of Plating Media into the tube, and gently pipet to resuspend the cells.

Note: Avoid repeated pipetting of thawed Cardiosight-S® to ensure maximum cell recovery.

8. Plating Cardiosight-S®

1. Confirm the number of viable cells using trypan blue exclusion method with a hemocytometer or an automated cell counter.
2. Calculate the final volume of provided Plating Media needed to obtain the desired cell plating density using the number of viable cells from the cell counting.
3. Gently shake the cell suspension and distribute the cells evenly into the coated cell culture plate.

Cell culture plate	6 well	12 well	96 well
Recommended cell numbers per well	2 X 10^6 cells	5 X 10^5 cells	1 X 10^5 cells
Plating Media per well	2 ml	1 ml	200 µl

Note: Adjust optimal cell number and media volume according to your research purposes.

4. After 1 day, confirm the state of cell attachment, replace with Maintaining Media and then incubate for 2 days.

9. Maintaining Cardiosight-S®

1. Equilibrate the Maintaining Media in cell culture hood to room temperature before use.
2. Culture the cells with Maintaining Media for 4 days after step 8 (Replace the Media every 2 days), and use the Cardiosight-S® for your research purpose.

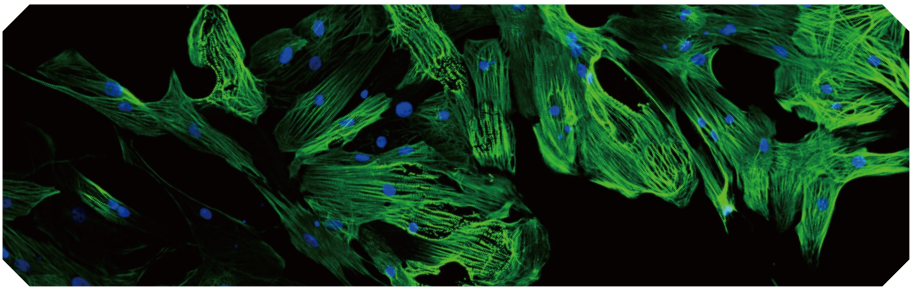
Cell culture plate	6 well	12 well	96 well
Plating Media per well	2 ml	1 ml	200 µl

Note: Cardiosight-S® can be aggregated by long term culture.

Note: We recommend to add the Maintaining Media slowly to ensure maximum viability and attachment

Licensed Patents

AJ No.	Country	Application No.	Patent No.
AJ001	Japan	2007-550210	5098028
AJ001	Japan	2008-131577	4183742
AJ001	Japan	2009-056747	4411362
AJ001	Japan	2009-056750	4411363
AJ001	Japan	2009-056748	5248371
AJ001	Japan	2009-056749	5467223
AJ001	Japan	2011-088113	5603282
AJ001	Japan	2013-167725	5943324
AJ001	China	200680048227.7	200680048227.7
AJ001	China	201010126185.2	201010126185.2
AJ001	China	201310015158.1	201310015158.1
AJ001	China	201410006027.1	Patent Pending
AJ001	Hong Kong	09102406.5	1125131
AJ001	Hong Kong	09103541.9	1125967
AJ001	India	3564/CHENP/2008	Patent Pending
AJ001	Korea	10-2008-7017015	10-1420740-0000
AJ001	Singapore	200804231-9	0143419



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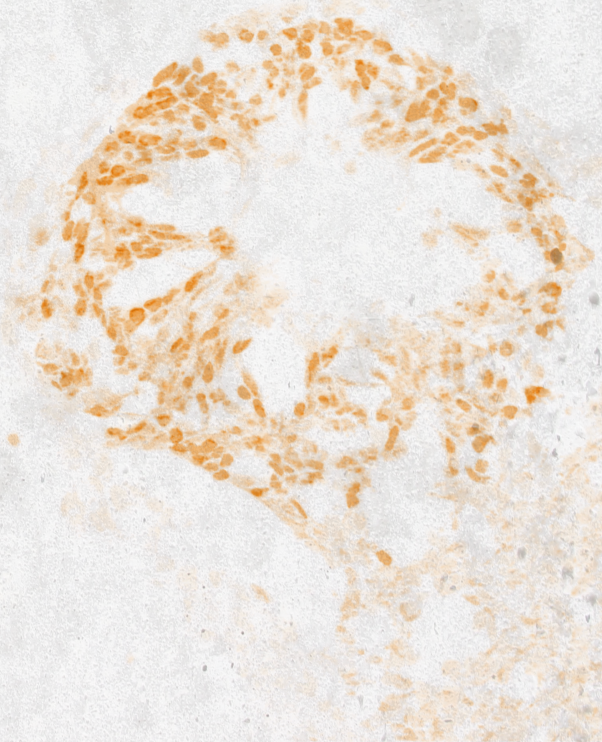

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