Handling and Culturing of Human iPSC-Cardiomyocytes on FLEXcyte 96 Well Plates

inno Vitro

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1. Introduction

This protocol outlines the handling and use of human iPSC-derived cardiomyocytes (hiPSC-CMs) from various commercial sources on FLEXcyte 96 (FLX-96) plates for cardiac contractility assessment.

The FLX-96 plates, developed by innoVitro, are ready-to-use, high-throughput platforms designed specifically for use with the FLEXcyte 96 system (Nanion Technologies). Each well contains a flexible membrane that serves as a substrate for the cells. These membranes are critical for measuring contractile function and must be handled with care. Disruption of the membrane will eliminate the well from performance in the FLEXcyte 96 system.

Before starting your experiment, please:

- Read this guide in full to ensure proper handling and optimal results.
- For questions about plate handling, contact: info@innovitro.de
- For FLEXcyte 96 software support, contact: support.ca@nanion.de

A downloadable PDF version of this guide is available at innovitro.de.

2. Workflow

Day	Morning	Afternoon
-1		Coating
0	Seeding	
1	Media change: maintenance medium (MM)	
2		
3	Media change: MM	
4		
5 -7	Media change: MM or alternative assay medium	Compound addition & acute measurement
7-13	Chronic measurements	Chronic measurements

3. Day -1

3.1. Important Notes

- FLX-96 plates are sterile and vacuum-sealed upon delivery. Always transfer the sealed plate into a sterile environment (e.g. laminar flow hood) before removing the plastic. If not used immediately, keep the vacuum seal intact and store the plate in a dry, dark place at room temperature to preserve sterility and functionality.
- Each well contains a fragile membrane protected by a standard lid (top) and a membrane guard (bottom). To avoid damage, ensure the membrane guard remains attached during handling. Remove it only when observing cells under a microscope or starting a measurement in the FLEXcyte 96 device.

3.2. Supplement Material

Reagents:

- Geltrex™ hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix (ThermoFischer Scientific A1569601)
- Optional: Fibronectin stock solution (1 mg/mL, e.g. Sigma Aldrich F1141)
- PBS -/- (e.g. GE Healthcare HyClone SH30028.02)

Disposable:

- FLX-96 plates
- Centrifuge tubes (50 mL)
- Serological pipettes (25mL)
- Reagent reservoirs (Integra CAT 4311)
- Pipette tips (1000 µL & 1250 µL)

Devices:

- Laminar flow hood
- Single channel adjustable pipette (e.g. 100-1000 µL)
- 12-channel adjustable pipette (100-1250 µL)
- Incubator (37 °C, 5% CO_2)

3.3. Coating Procedure

1. Prepare the ECM working solution by transferring 2.75 mL of Geltrex™ Ready-To-Use into a sterile centrifuge tube. Add 8.25 mL of DPBS and mix gently.
   (optional: fibronectin coating) Prepare 11 mL of fibronectin solution by diluting 110 µL of 1 mg/mL fibronectin stock in 10.89 mL DPBS to achieve a 10 µg/mL final concentration. Mix gently.

2. Transfer the coating solution into a sterile reagent reservoir. Using a 12-channel pipette, dispense 100 µL into each well of the FLX-96 plate.

3. Cover the FLX-96 plate with its lid and incubate at 37 °C for 16 – 24 hours.

4. Day 0

4.1. Important Notes

- Follow the cell manufacturer's instructions closely to ensure optimal cell performance on the FLX-96 plate. Refer to Table 1 for recommended seeding densities to achieve a synchronously beating syncytium. For consistent and reliable cell counts, we recommend using a manual counting chamber.

4.2. Supplement Material

Reagents:

- Cell culture medium
- HiPSC-derived cardiomyocytes

Disposable:

- Coated FLX-96 plates
- Centrifuge tubes (50 mL)
- Serological pipettes (25mL)
- Reagent reservoirs (Integra CAT 4311)
- Pipette tips (1000 µL & 1250 µL)

Devices:

- Laminar flow hood
- Single channel adjustable pipette (e.g. 100-1000 µL)
- 12-channel adjustable pipette (100-1250 µL)
- Centrifuge (50 mL tubes)
- Water bath (37 °C)
- Incubator (37 °C, 5% CO_2)
- Vacuum aspiration system

4.3. Recommended cell numbers for optimal seeding densities

Recommended seeding densities are provided in the table below. These values are based on internal experience and may differ from the guidelines supplied by the respective commercial cell provider. To ensure full plating efficiency, we recommend preparing the cell suspension with an excess of 15 % both for the total cell number as well as the total volume.

When working with a new or unlisted cell type, it is recommended to start with a seeding density approximately 30% higher than the provider's suggestion and adjust as needed based on observed cell behavior.

Table 1

Cell Type	Manufacturer	Seeding Density per Well
iCell CM^2, 01434	Fujifilm Cellular Dynamics Inc.	6 x 10^4 cells per well
iCell Cardiomyocytes DCM LMNA L35P, 01016	Fujifilm Cellular Dynamics Inc.	5 x 10^4 cells per well
iCell Cardiomyocytes DCM LMNA L35P corrected, 01016	Fujifilm Cellular Dynamics Inc.	5 x 10^4 cells per well
axoCells atrial Cardiomyocytes, ax2518	Axol Bioscience Ltd.	6 x 10^4 cells per well
axoCells Ventricular Cardiomyocytes, ax2508	Axol Bioscience Ltd.	6 x 10^4 cells per well
Cardiosight®-S	Nexel Ltd.	5 x 10^4 cells per well
Celo.Cardiomyocytes	Celogics Inc.	5 x 10^4 cells per well
YBLiCardio	Yashraj Biotechnologies Ltd.	7,5 x 10^4 cells per well
Ncytes	Ncardia B.V.	4 x 10^4 cells per well

4.4. Seeding of human iPSC-CMs into FLX-96 plate

1. Thaw the cells according to manufacturer's guidelines.
2. Count the cells with a manual counting chamber and adjust the cell number in the provided Plating Medium according to table 1.
3. Transfer the cell suspension (11mL total) into a sterile reagent reservoir.
4. Remove the coating solution from the wells with a vacuum aspiration system.
5. Pipette 100 µL of the cell suspension to each well of the FLX-96 plate using a 12-channel pipette.
6. Immediately after cell seeding, transfer the FLX-96 plate into the incubator and let the cells settle overnight.

5. Day 1

5.1. Important hints

- A full medium exchange with Maintenance Medium is recommended 18–24 hours after seeding. Afterward, each well should contain 200 µL of medium. Frequency of medium changes should follow the cell manufacturer's recommendations.
- For manual medium changes, remove medium row by row using a single-channel vacuum aspirator. Do not aspirate the entire plate at once to avoid cell drying. Add fresh and warm medium immediately after aspirating up to four rows. Keep the plate on a flat surface and hold the 12-channel pipette at a 45° angle to minimize stress on the cell layer.
- For automated medium changes, the entire plate can be processed at once. The high speed of the robotic system prevents the cells from drying during the exchange.

5.2. Supplementary material

Reagents:

- Minimum of 22 mL Maintenance Medium

Disposables:

- Serological pipette (e.g. 25 mL)
- Pipette tips (1250 µL)
- Reagent reservoir

Devices:

- Laminar flow hood
- 12-channel pipette (100-1250 µL)
- Water bath (37 °C)
- Incubator (37 °C, 5% CO_2)

5.3. Medium exchange of FLX-96 plates

1. 18-24 h after seeding, warm at least 22 mL of Maintenance Medium for one FLX-96 plate to 37°C and transfer the warm medium into a sterile reagent reservoir.
2. Hold the FLX-96 plate in a 45°angle and aspirate four rows of the FLX-96 plate (A-H) with a single channel vacuum aspiration system.
3. Dispense 200 µL fresh medium into each well using a 12-channel pipette. Leave the FLX-96 plate on a flat surface and hold the 12-channel pipette in a 45° angle to avoid maximum stress for the cell layer.
4. Proceed with the next four rows of the FLX-96 plate (E – H) and repeat steps 2 and 3.
5. Immediately after medium exchange transfer the FLX-96 plate back into the incubator.

6. Day 5 – 7

6.1. Important hints

- Perform a final medium change 4–6 hours before starting measurements to maintain stable nutrient levels for optimal cardiomyocyte performance.
- Use serum-free medium during compound measurements when analyzing positive inotropic effects. For long-term experiments, follow the cell manufacturer's guidelines on medium usage.
- Prepare a measurement plan in advance and perform a baseline recording (recommended: at least 3 sweeps with 5 min intervals) on the FLX-96 plate in the FLEXcyte 96 device immediately before compound addition.
- Prepare compounds as 4× concentrated solutions; after removing 50 µL of the medium from each well, add 50 µL per well (¼ of total volume) to achieve the final 1× working concentration. Add compounds quickly to prevent plate cooling. Perform the addition directly in the FLEXcyte 96 device to avoid mechanical stimulation of the cells due to plate handling.

6.2. Supplementary material

Reagents:

- 22+ mL complete culture medium or alternative assay medium provided by cell manufacturer
- 6+ mL iCell Maintenance Medium or alternative assay medium for compound preparation
- Compounds

Disposables:

- Serological pipette (e.g. 25 mL)
- Pipette tips (1250 µL)
- Reagent reservoir
- 96 deep well plate (for compound preparation)

Devices:

- Laminar flow hood
- FLEXcyte 96 device (Nanion Technologies)
- 12-channel pipette (100-1250 µL)
- Water bath (37 °C)
- Incubator (37 °C, 5% CO2)
- Vacuum aspiration system

6.3. Final medium exchange before compound addition

1. Perform a final medium change 4 – 6 h before compound addition.
2. Warm at least 22 mL of Maintenance Medium or alternative assay medium for one FLX-96 plate and transfer the warm medium into a sterile reagent reservoir.
3. Hold the FLX-96 plate in a 45°angle and aspirate four rows of the FLX-96 plate (A-H) with a single channel vacuum aspiration system.
4. Dispense 200 µL fresh medium into each well using a 12-channel pipette. Leave the FLX-96 plate on a flat surface and hold the 12-channel pipette in a 45° angle to avoid maximum stress for the cell layer.
5. Proceed with the next four rows of the FLX-96 plate (E – H) and repeat steps 3 and 4.
6. Immediately after medium exchange transfer the FLX-96 plate back into the incubator.

6.4. Compound addition and analysis

1. Prepare 4× concentrated compound working solutions in a sterile 96 deep-well plate under the laminar flow hood. Incubate the plate for at least 1 hour to equilibrate with FLX-96 plate conditions.

2. Place the FLX-96 plate in the FLEXcyte 96 device 1 hour before baseline measurement. Perform 3 baseline sweeps at 5-minute intervals shortly before compound addition.

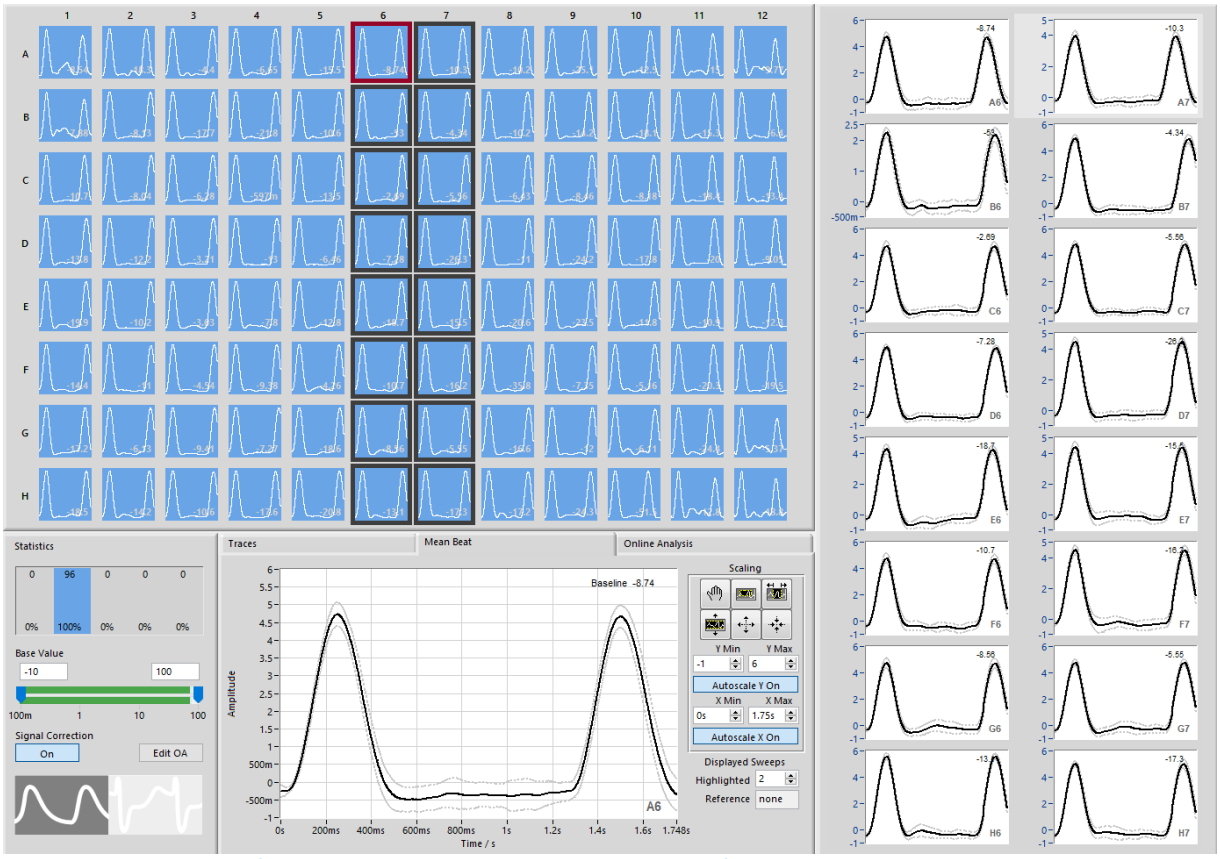

Figure 1: Reference baseline measurement before compound application

3. Remove 50 µL of medium from each well of the FLX-96 plate.

4. Add 50 µL of the 4× concentrated compound solution to each well as per your measurement plan.

5. Start the measurement in the FLEXcyte 96. Measurement frequency and intervals may vary depending on whether you are performing acute or chronic analysis.

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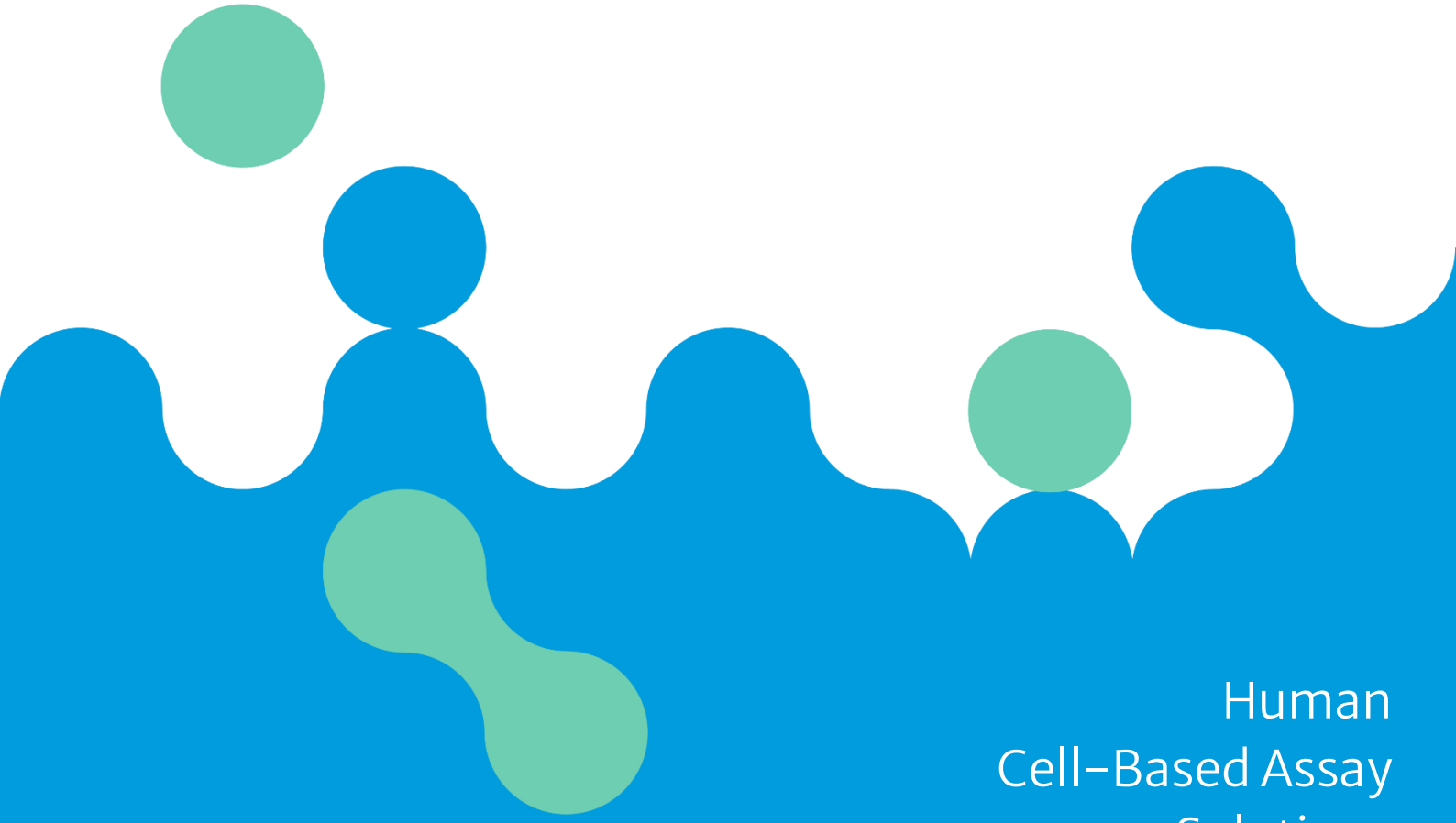

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