Handling Guide

FLX-96 Plate

Handling guide for FLEXcyte 96 (FLX-96) plates used for contractility assays with human iPSC-derived cardiomyocytes.

TOC

1. Introduction

The FLEXcyte 96 plates (FLX-96) are designed for high throughput cardiac contractility measurements using the FLEXcyte 96 system, an add-on system for the CardioExcyte 96 device provided by Nanion Technologies.

FLX-96 plates are ready-to-use and should always be handled with care. Each of the wells contains a flexible membrane as substrate for the cells, disruption of the membrane will eliminate the well from performance in the FLEXcyte 96. Please read the entire user guide before you start your experiment and contact support@innovitro.de in case of questions regarding handling of the FLX-96 plate or support.cellular.networks@nanion.de for questions regarding the FLEXcyte 96 software.

The cardiomyocytes used for FLX-96 plates are obtained from human induced pluripotent stem cell (iPSC) and can be purchased by a variety of stem cell manufacturers. According to the manufacturer, differences in cell handling may occur (e.g. cryopreserved or pre-cultured cells upon delivery, medium or high cell numbers for seeding). Please read the manufacturer's cell handling guide carefully before you start your experiment with the FLX-96 plate.

Depending on the aim of experiment, a serum-free buffer might be of need for compound addition and time of measurements. E.g. when positive inotropic effects of cardiomyocytes are of main interest. A validated serum-free buffer can be purchased with the FLEXcyte 96 well plates from innoVitro and should always be equilibrated in the incubator the day before the start of the experiment.

Helpful clues for every step are provided at the beginning of each chapter. Please read these hints carefully to obtain optimal results with FLEXcyte 96 plates.

You will find a PDF version of this Handling Guide on www.innovitro.de

2. Workflow

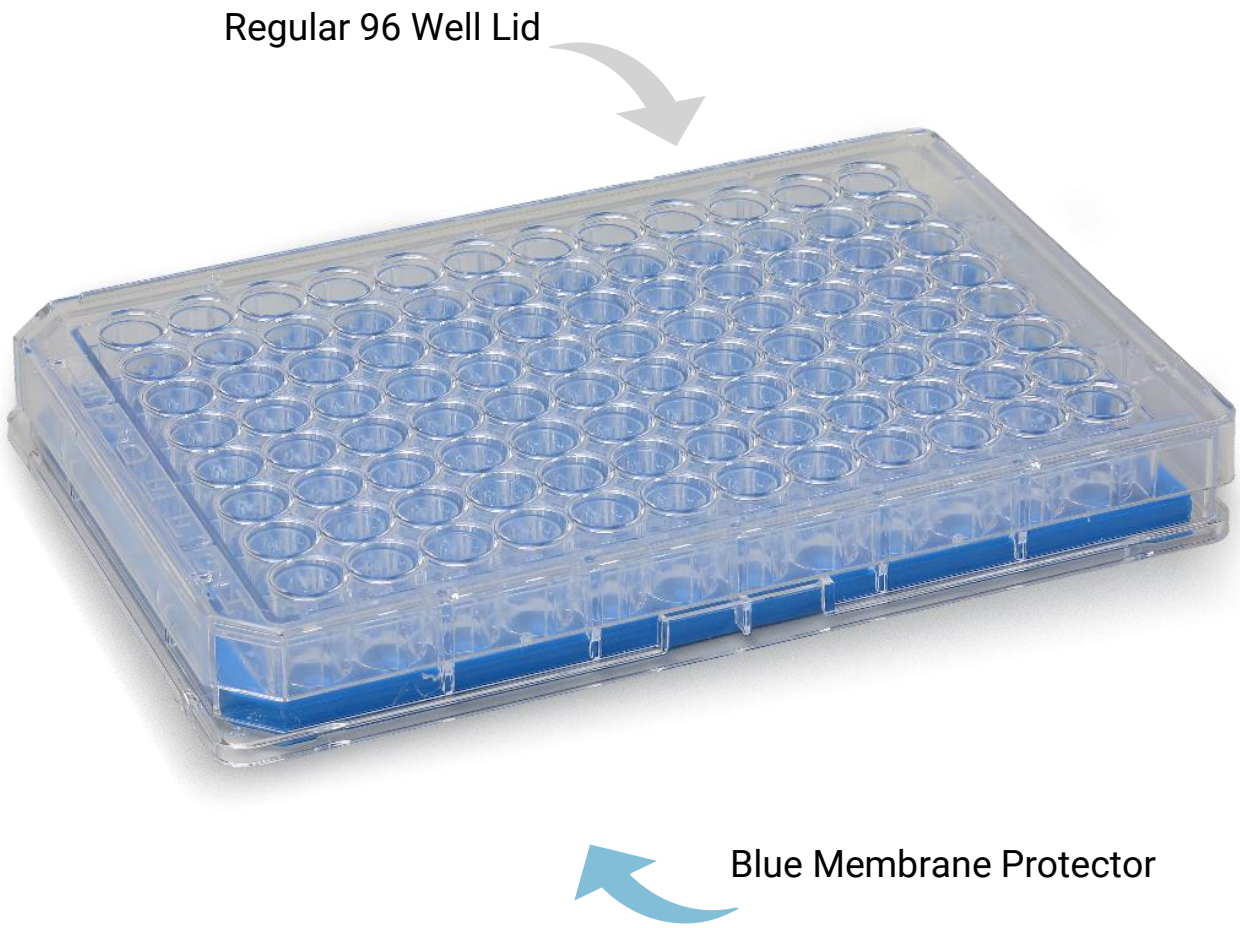
- **Day 0**: FLX-96 preparation / coating / seeding
- **Day 1**: FLX-96 medium exchange
- **Day 2 – Day 5-7**: FLX-96 maintenance
- **Day 5-7**: FLX-96 compound addition and analysis

Notes

3. Day 0 - Handling of FLX-96 plates upon arrival and before use

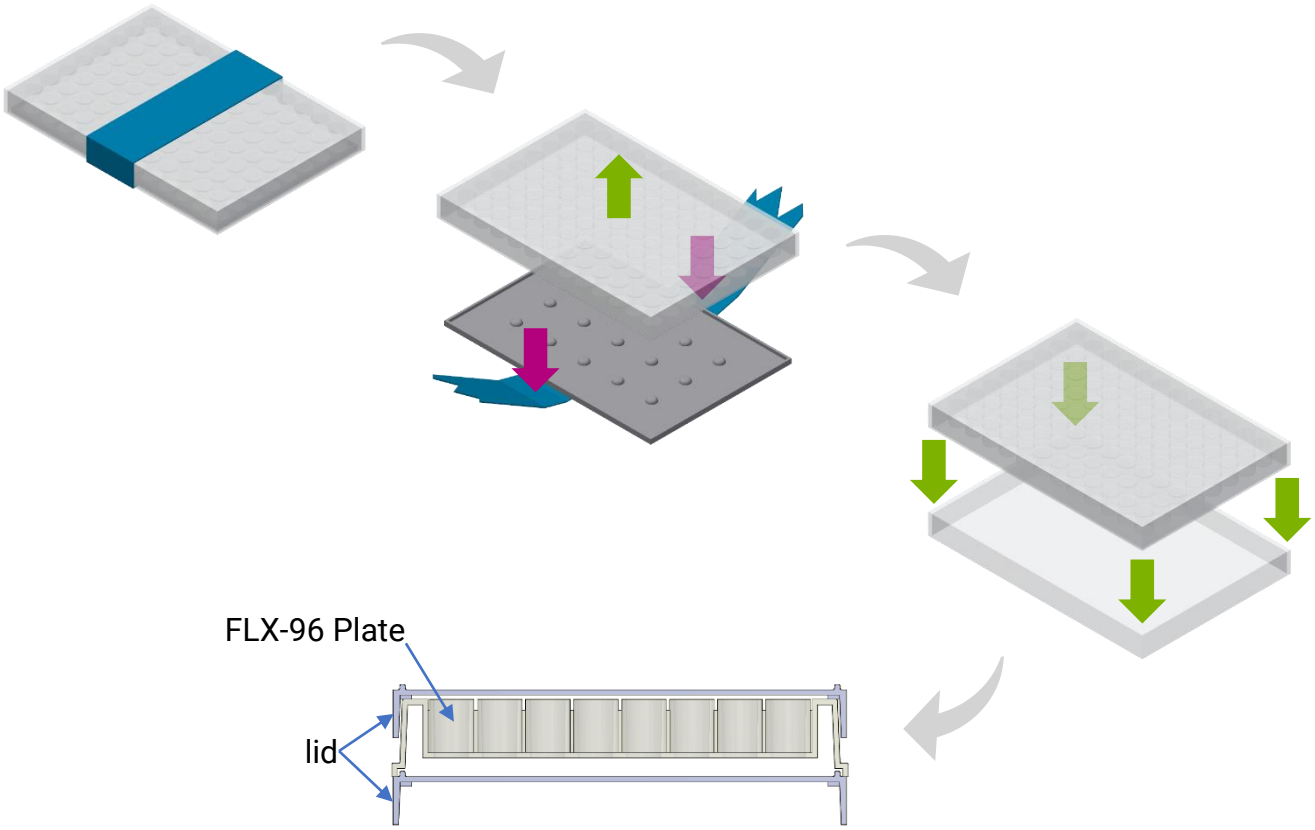
3.1 Important hints upon arrival of FLX-96 plates

- FLX-96 plates are sterile and covered in vacuum-sealed plastic upon arrival. Please transport the sealed FLX-96 plate to a sterile environment (e.g. laminar flow hood) before removal of the plastic to avoid contamination before use. If the plates are not used straight away, do not remove the vacuum-sealed plastic and store the plates in a dry and dark environment at room temperature.
- The bottom of each FLX-96 well is covered with a very thin membrane which may be damaged if touched from inside or outside. Upon arrival, the FLX-96 plate is protected from above with a regular 96 plate lid and from below with a blue membrane protector. Before you start your experiment, make sure to exchange the blue membrane protector with a regular 96 plate lid (additionally provided with the FLX-96 plate). See step 3.2 (Preparation of FLX-96 plates before use) for detailed description how to handle FLX-96 plates upon arrival.



3.2 Preparation of FLX-96 plates before use

1. Transport the sterile and vacuum-sealed FLX-96 plate into a sterile environment, e.g. a laminar flow hood.
2. Unpack the additionally supplied lid and place it on a flat surface.
3. Open the vacuum-sealed packaging and remove it from the FLX-96 plate.
4. Put the FLX-96 plate together with the membrane protector on a flat surface.
5. Break the banderole and carefully lift the FLX-96 plate together with the upper lid (green arrow) while holding the membrane protector (purple arrow) down at the remains of the banderole.
6. Place the FLX-96 plate upon the additional lid by stacking the plate onto the lid. Do not turn the lid upside down to avoid disruption of the membranes. Keep the FLX-96 plate stack onto the lid until measurements are performed in the FLEXcyte 96. (Approx. 5-7 days after seeding)



4. Day 0 - FLX-96 plate coating with fibronectin

4.1 Supplementary material for FLX-96 plate coating

Reagents:

- Fibronectin stock solution (1 mg/mL, e.g. Sigma Aldrich F1141)
- DPBS with Ca^{2+} and Mg^{2+} (e.g. GE Healthcare HyClone SH304264.01)
- Complete culture medium (optional: plating medium, please check cell manufacturer's guidelines before you start)

Disposable:

- FLX-96 plates
- Centrifuge tubes (50 mL)
- Serological pipettes (25 mL)
- 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
- *Optional: VIAFLO ASSIST (Integra 4500)*
  VIAFLO 12 channel Pipette (Integra 4634)

4.2 Procedure

1. Prepare 11 mL fibronectin coating solution in a sterile centrifuge tube by diluting 110 µL fibronectin stock solution (1 mg/mL) in 11 mL of DPBS with Ca^{2+} and Mg^{2+}, resulting in a 10 µg/mL fibronectin working solution. Mix the solution carefully.
2. Transfer the coating solution into a sterile reagent reservoir. Pipette 100 µL of the coating solution to each well of the FLX-96 Plate using a 12-channel pipette.

   2. (optional) When using a VIAFLO ASSIST with a 12-channel pipette, transfer the coating solution into a sterile reagent reservoir placed in the VIAFLO ASSIST, use program "ADD100µL" and start the coating procedure.
3. Visually confirm that all membranes are completely covered by the solution.
4. Place the lid back on the FLX-96 and incubate the plate for 3 h at 37 °C.

5. Day 0 - Seeding of hiPSC-derived cardiomyocytes into FLX-96 plates

5.1 Important hints before seeding of FLX-96 plates

➔ Cells may be purchased cryopreserved or pre-cultured depending on the manufacturer. Also, different media for seeding or compound addition might be provided by the manufacturer. Please read the cell manufacturer instructions carefully and follow recommendations for cell handling to ensure an optimal performance of the cells on the FLX-96 plate.

➔ According to your choice of stem cell manufacturer, different seeding densities of cardiomyocytes are required to achieve a synchronous beating syncytium. Please refer to table 1 for seeding densities according to your chosen cardiomyocytes.

➔ We recommend a manual counting chamber for consistent cell counting results.

➔ Depending on the number of compounds tested per FLX-96 plate, you might not need to seed the entire FLX-96 plate. Please refer to the compound example layouts per FLX-96 plate supplemented at the end of this document before seeding the cells.

Notes

5.2 Supplementary material for FLX-96 plate seeding

Reagents:

- Complete culture medium (provided by cell manufacturer)
- Plating Medium (only when recommended and provided by cell manufacturer)
- hiPSC-derived cardiomyocytes (cryopreserved or pre-seeded)

Disposables:

- Fibronectin-coated FLEXcyte 96 Plate (from Step 1.4)
- Centrifuge tubes (50 mL)
- Reagent reservoir
- 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
- *Optional: VIAFLO ASSIST (Integra 4500)*
  VIAFLO 12 channel Pipette (Integra 4634)

Notes

Table 1. Cell numbers for optimal cardiomyocyte seeding densities

Cell Type Manufacturer	**Cell state before seeding**	**Cell number / well**	**Plating volume / well**	**Total cell number / volume per FLX-96 plate**
iCell Cardiomyocyte² Cellular Dynamics	cryopreserved	1 x 10^5	100 µL	11 Mio / 11 mL
Ncytes Ncardia	pre-seeded	3 x 10^4	100 µL	3.3 Mio / 11 mL
CardioSight-S Nexel	cryopreserved	5 x 10^4	100 µL	5.5 Mio / 11 mL
Ventricular cardiomyocytes Axol Biosciences	cryopreserved	5 x 10^4	100 µL	5.5 Mio / 11 mL

To ensure full plating efficiency, we recommend to prepare cell suspension with an excess of 15% both for the total cell number as well as the total volume. This results in a total volume of the cell suspension of 11 mL.

Notes

5.3 Procedure

1. Thaw the cells according to manufacturer's guidelines.
2. Count the cells with a manual counting chamber and adjust the cells according to table 1 (right column).
3. Transfer the cell suspension (11 mL total) into a sterile reagent reservoir.
4. Remove the fibronectin 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.

   5. (optional) When using a VIAFLO ASSIST with a 12-channel pipette, transfer the cell suspension into a sterile reagent reservoir placed in the ViaFLO Assist, use program "CELLS_ADD100µL" and start the seeding procedure.

6. Immediately transfer the FLX-96 plate into the incubator and let the cells settle over night.

6. Day 1 – approx. Day 6 - Medium exchange of FLX-96 plates

6.1 Important hints before medium exchange of FLX-96 plates

- ➔ We recommend a complete medium exchange with complete culture medium 18-24h after seeding.
- ➔ When medium change is performed manually: Remove the medium carefully row-wise with a single channel vacuum aspiration system. Four rows can be aspirated at a time before adding fresh medium. Do not remove the medium of the entire FLX-96 plate at once before adding fresh medium to avoid drying of the cells. Leave the FLX-96 plate on a flat surface when adding fresh medium and hold the 12-channel pipette in a 45° angle against the wall to avoid direct stress for the cell layer.
- ➔ When medium change is performed automatically with a pipette robot (e.g. INTEGRA VIAFLO ASSIST): The medium change can be performed at once for the entire plate, due to the high speed of the assist the cells will not dry out during the procedure.
- ➔ After medium change, each well should contain a final volume of 200 µL.
- ➔ Recommendations regarding the medium exchange cycles should be obtained from the cell manufacturer.

6.2 Supplementary material for FLX-96 plate medium exchange

Reagents:

- Minimum of 22 mL complete culture 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)
- *Optional: VIAFLO ASSIST (Integra 4500)*

  VIAFLO 12 channel Pipette (Integra 4634)

Notes

6.3 Procedure

1. 18-24 h after seeding, warm at least 22 mL of maintenance medium for one FLX-96 plate.
2. Transfer the warm maintenance medium into a sterile reagent reservoir.
3. Aspirate the medium carefully with a single channel vacuum aspiration system to avoid disruption of the cell layer or membrane. Hold the FLX-96 plate in a 45°angle and aspirate four rows of the FLX-96 plate (A-H).

   3. (optional) When using a VIAFLO ASSIST with a 12-channel pipette, transfer the fresh medium into a sterile reagent reservoir and leave it right next to the VIAFLO ASSIST. Place an empty reagent reservoir in the VIAFLO ASSIST, use program "REMOVE100µL" and perform medium removal twice. Afterwards exchange the reagent reservoir containing the waste medium with the reagent reservoir containg the fresh medium and dispense the fresh medium with program "ADD100µL". Perform this step twice again to reach the final volume of 200 µL per well. Skip step 4 and 5 and proceed with step 6.

4. Dispense 200 µL 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 transfer the FLX-96 plate back into the incubator.

Notes

7. Day 5 - Day 7 - Compound addition and analysis

7.1 Important Info before compound addition to FLX-96 plates

- ➔ Perform a last medium change 4-6 h before starting your measurements to keep nutrition levels stable for best cardiomyocyte performance.
- ➔ If positive inotropic effects are the main focus, we recommend serum-free medium during compound measurements. Please equilibrate the serum-free medium with the cap slightly open over night in the incubator the DAY BEFORE compound addition.
- ➔ If long-term measurements are performed (max. 5 days), we recommend serum containing complete culture medium during compound measurements.
- ➔ Have your measurement plan ready before you start your experiment. Use Figure 1. (see appendix) as 96 well plate design template to set up your compound analysis plan.
- ➔ Prepare compounds in a 4x concentrated manner. Compound addition is performed with ¼ (50 µL) of the total medium per well, hence the final concentration will dilute to 1x concentrated per well.
- ➔ Perform a baseline measurement of the FLX-96 plate in the FLEXcyte 96, 15 min BEFORE you add the compounds. We recommend 3 sweeps for a good reference baseline.
- ➔ Compound addition should be performed quickly to avoid temperature decrease of the plate. When using a FLEXcyte 96 benchtop device: compound addition is performed in the device.

Notes

7.2 Supplementary material for compound addition and analysis

Reagents:

- 22+ mL complete culture medium *or* serum-free buffer for final medium change
- 6+ mL complete culture medium *or* serum-free medium for compound preparation
- Compounds for your analysis

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% CO_2)
- Vacuum aspiration system
- *Optional: VIAFLO ASSIST (Integra 4500)*

  VIAFLO 12 channel Pipette (Integra 4634)

Notes

7.3 Procedure

1. Perform a final medium change 4-6 h before compound addition as described in 6.3.
2. Prepare your compound working solution in the laminar flow hood using a sterile regular 96 deep well plate. The working solution per compound should be 4x concentrated. Transfer the 96 deep well plate containing the compound solution for at least 1 h into the incubator to adjust it to the same condition as the FLX-96 plate.
3. Transfer the FLX-96 plate into the FLEXcyte 96 device and perform a baseline measurement 15 min before you add the compounds. We recommend 3 baseline measurements (sweeps) in 5 min intervals.
4. Remove 50 µL medium of each well of the FLX-96 plate.
5. Add 50 µL of the 4x concentrated compound solution into the FLX-96 plate, according to your measurement plan.
6. Start your measurements in the FLEXcyte 96. The number and intervals of measurements may differ dependent on acute or chronic analysis.
   Please refer to support.cellular.network@nanion.com for support regarding FLEXcyte 96 software and analysis.

Notes

8. Appendix

Figure 1. 96 well plate template

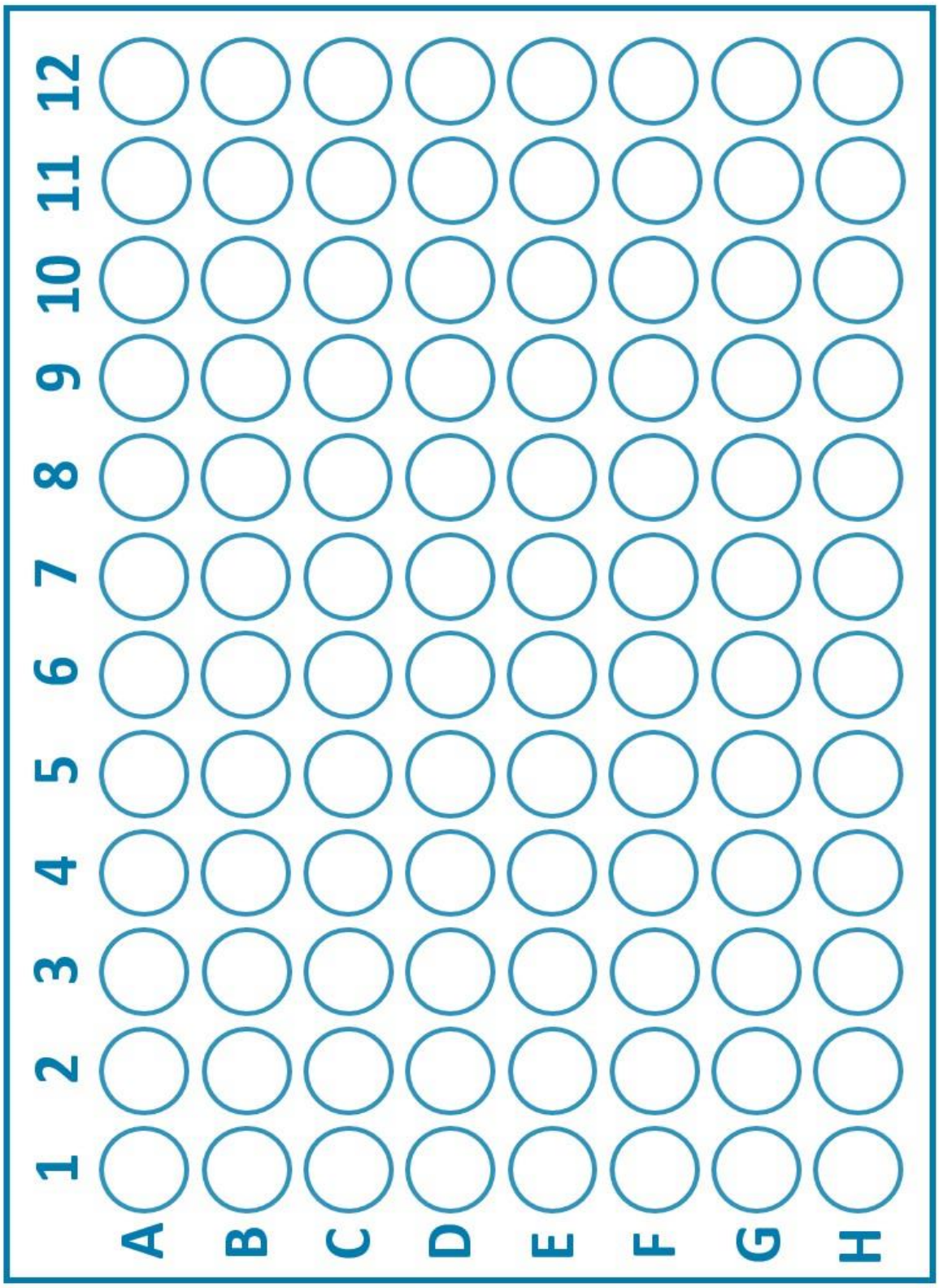
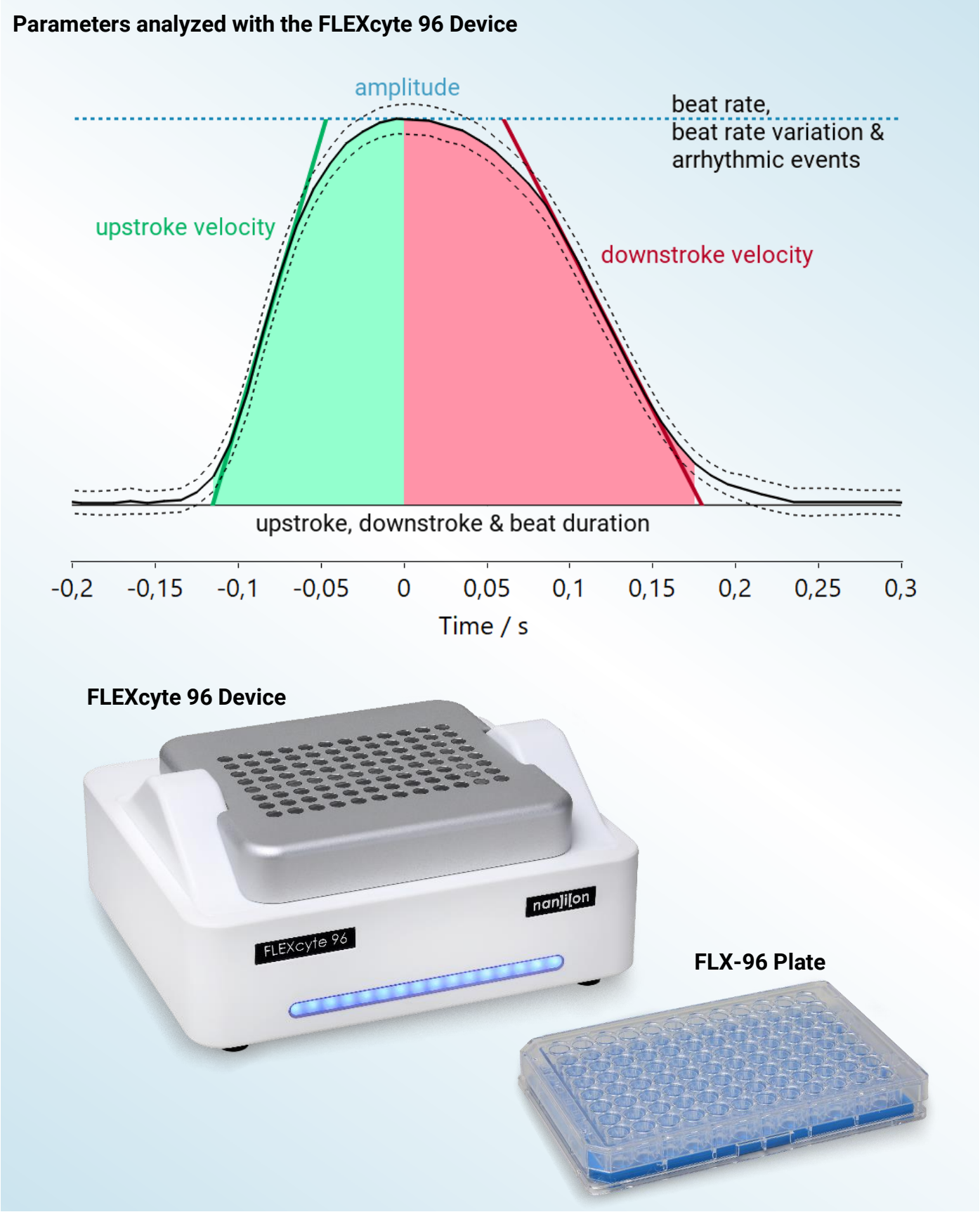


Figure 2. Multiparametric analysis of cardiac contraction with the FLEXcyte 96 device and FLX-96 plates



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