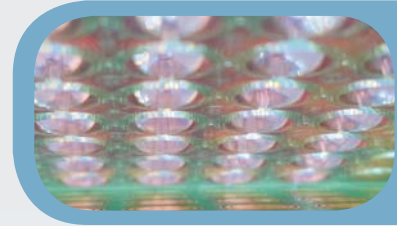


The FLEXcyte 96.

True contractility.



Power tool
for contractility
studies



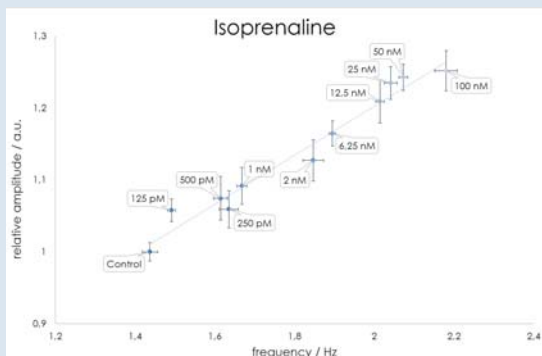
The FLEXcyte 96

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FLEXcyte 96 True contractility

- Real contractility measurements
- Physiological mechanical conditions
- Leveraging mature cardiac phenotype
- Add-On for CardioExcyte 96
- Non-invasive & label-free measurements
- Monitoring acute and chronic effects
- Optical pacing options
- Integrated liquid handling system
- Real-time access to beating parameters
- Incubation System
- Cost efficient consumables
- Black plates for optical stimulation
- Ideal for cardiotox and efficacy studies
- Outstanding data analysis software

Isoproterenol effects



Positive force-frequency-relation in iCell CM² after treatment with isoproterenol. No additional external stimulation is needed.

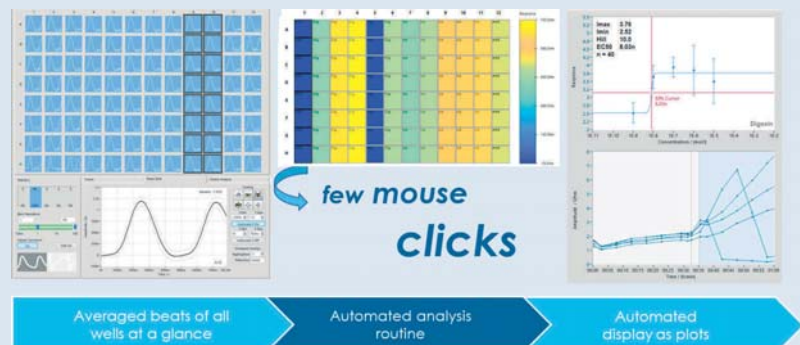
Simulating native human cardiac environment in 96 wells

The FLEXcyte 96 brings in vitro contractility measurement to a new dimension. By providing a well-defined, flexible substrate to the cells, the system leverages mature responses of stem cell-derived cardiomyocytes to pharmacological compounds. Based on the CardioExcyte 96 platform, the FLEXcyte 96 add-on is a turn-key system for structural and functional cardiotoxicity screening as well as efficacy testing. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) display positive inotropic responses without additional stimulation.

The assay has been validated with stem cell-derived cardiomyocytes from several providers (NCardia, Cellular Dynamics International, Axol and NEXEL).

Experimental workflow with the FLEXcyte 96

Typical workflow on the FLEXcyte 96. An overview of all wells recorded is the first step in the analysis process. Parameters such as beat amplitude and arrhythmia events detected. Finally, average concentration response curves for all wells receiving a particular compound are pooled and displayed, along with timecourse plots for individual wells.



DataControl 96 Software Package.

Versatile data analysis & data handling.

The FLEXcyte 96 control software supports online analysis of beating parameters. The unique Mean Beat Function automatically visualizes the average of beat traces from one well, enveloped by the standard deviation, indicating the consistency of the beat pattern within the recorded well, i.e. if the cellular network is synchronously beating or not.

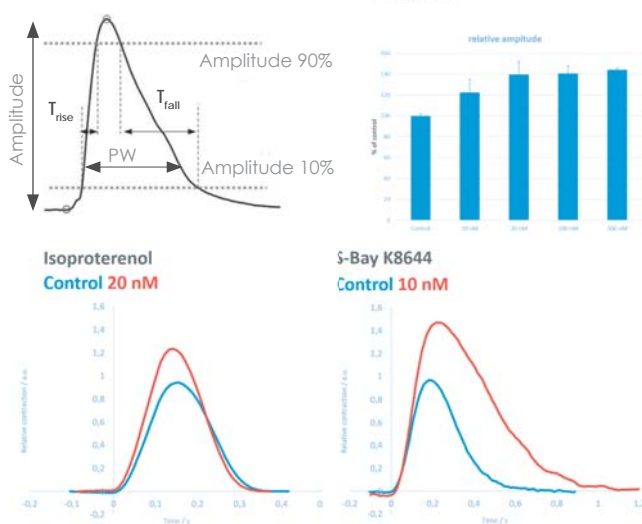
The control software is intuitive and easy-to-use, at the same time very powerful in terms of experimental set-up and online visualization of the recorded data.

The **DataControl 96** software package is an independent software package to load and analyze data recorded on the FLEXcyte 96. Customized export formats of analysis results are integrated.

Supporting standardized processes, analysis templates can be saved and re-loaded. Normalization methods, for example, provide control group normalization or pooling of data across many wells. Compound effects of individual wells or the average concentration response curves of all wells can be easily inspected.

- Easy-to-use yet very powerful software
- *Ad* and *post hoc* data analysis possible
- Raw data traces, results and arrhythmia detection all on one single screen
- Mean beat analysis for improved data quality and consistency
- Instant recalculation of data upon parameter changes
- Highly customizable exporting to databases
- Broad range of QC options
- Templated data analysis allows for results within seconds
- Data can be analyzed on multiple computers making inter-department collaborations easy

Geometric beat analysis



Positive inotropic responses on calcium channel agonist S-Bay K8644 and shape changes of mean beats in the presence of 20 nM Isoproterenol and S-Bay K8644, iCell CM².

FLEXcyte 96 Online Analysis

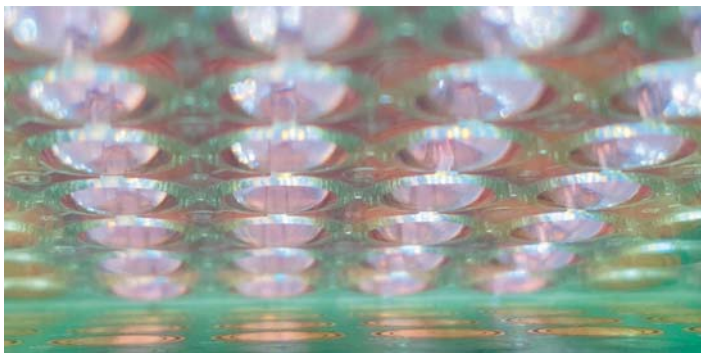
- Real-time geometric beat analysis
- Signal read out: Beat rate/regularity, amplitude, integrals, rise time, fall time, pulse width etc.
- Sensor temperature read out
- Compound Arrhythmia Factor analysis based on arrhythmic events
- *Ad* & *Post hoc* analysis of data

Elasticity is key

Cardiomyocytes constantly sense their mechanical environment through multiple cellular processes, including deformable adaptor proteins and mechanosensitive ion channels. When cultured on overly stiff substrates like glass or plastic, cardiomyocytes respond with drastic transcriptional and metabolic dysregulation.

With a thickness of less than 10 μm and sophisticated surface modification, the polydimethylsiloxane (PDMS) membranes of the FLEXcyte 96 plates combine both physiological elasticity of native

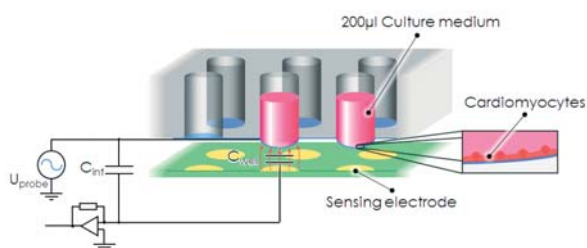
human heart tissue and strong mechanical support. The plates come in a standard 96-well format, allowing for automated liquid handling and easy integration into your lab procedures. After 7 days of culture, the cells have formed a biohybrid material with the membranes and are ready for assay. For further maturation in the physiological environment provided by the FLEXcyte 96 plates, the cells can be cultured for up to 4 weeks.



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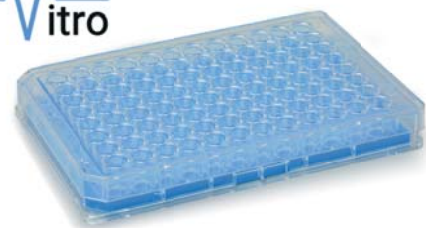
CIPA

High-precision measurement of real contractility



While being statically deflected downwards by the weight of the culture medium, synchronous beating of the hiPSC-CMs results in dynamic deflection changes of the cell-membrane biohybrids (up and down movement). Our proprietary capacitive distance sensors co-developed with innoVitro allow for the acquisition of real contraction under physiological mechanical conditions in 96 wells at a time with high temporal (10 ms) and spatial (< 10 μm) resolution.

inno
vitro



FLEXcyte 96 Sensor Plate

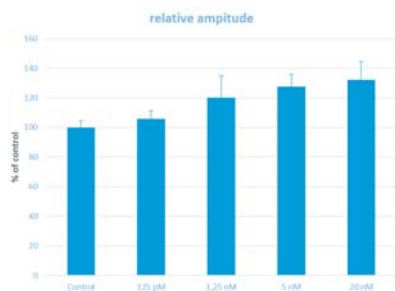
- Standard 96-well plate format
- optional black plates for optical stimulation experiments
- Supports recordings from 2D and 3D structures
- produced and certified by innoVitro, Germany

FLEXcyte 96 Specifications:

readout:	mechanical contraction
resolution & bandwidth:	1-10 ms, 0-500 Hz
parameters:	amplitude, durations, slopes, integrals, frequency, arrhythmia
temperature sensing:	multi-sensor temperature control
validated induced human pluripotent stem cells:	iCell CM2®, AXOL CM, Cardiosight-S®
validated stem cell derived cells:	Cor.4U®
parallel recording channels:	96
stand-alone system:	integrated Incubation System
automated liquid handling:	Integra Viaflow Assist
cardiac cell pacing:	optical (optional)
plates available:	standard plates (transparent); optical stimulation plates (black)

Positive inotropic responses on beta-adrenergic stimulator isoproterenol can be detected down to the pico-molar range

Isoproterenol



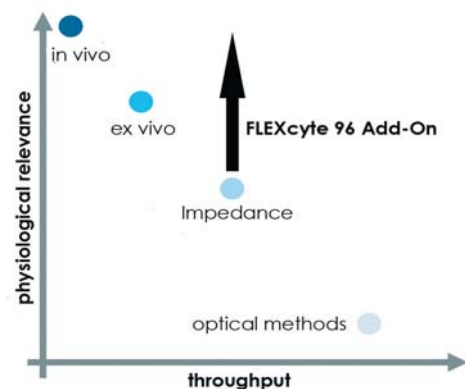
Benchmark for a mature phenotype of hiPSC-CMs

Stem cell-derived cardiomyocytes are suspected to be immature as they do not respond physiologically on certain compounds, e.g. showing negative inotropic response upon beta-adrenergic stimulation. The culture substrate of the FLEXcyte 96 allows the cells to contract like they would do in native human heart tissue. Therefore, hiPSC-CMs react much more physiological and relevant than in assays based on stiff substrates. Positive inotropic effects can be displayed without additional external stimulation. In this way, the CardioExcyte 96 with a FLEXcyte 96 is an excellent choice offering new strategies for efficient and reliable safety and cardiotoxicity screening.

The hybrid approach:

Combining viability, electrophysiology and true contractility

The CardioExcyte 96 provides access to high resolution contractility and EFP data from the same cells. The FLEXcyte 96 add-on combines physiological relevance while providing an environment that reflects the mechanical properties of real human cardiac tissue. The 96-well format is compatible to standard lab automation systems and minimizes sample volumes compared to ex vivo and in vivo approaches. Aspects such as force-frequency relation are subject of current scientific discussions and require an assay technology that can accurately quantify them.



Leveraging maturity of iPSC-CM cells by FLEXcyte 96 assays.

"Human iPSC-derived cardiomyocytes are the future of safe and efficient drug development. The FLEXcyte technology provides a tool for functional cardiac toxicology and efficacy screening on these cells. Force-frequency relation are the subject of current scientific discussions and require an assay technology that can accurately quantify them and we meet this need with the FLEXcyte 96."

Peter Linder, CTO, innoVitro, Germany

"Screening services on the FLEXcyte 96 deliver functional measurements at physiological mechanical conditions. Acute or chronic (up to 5+ days) cardiac toxicity or efficacy assessment is possible, with comprehensive endpoints from human iPSC-derived cardiomyocytes. We at innoVitro are proud to offer high quality screening services using Nanion's FLEXcyte 96 instruments as a CRO."

Matthias Gossman, CEO, innoVitro, Germany

"CardioExcyte 96 is an easy-to-use system, providing impedance-based and MEA-like cardiac safety data from a diversity of stem cell-derived cardiomyocytes. In a collaborative effort between innoVitro and Nanion Technologies, the technology using flexible substrates was fused with the CardioExcyte 96 platform resulting in the FLEXcyte 96 system. The FLEXcyte 96 system has the potential to scale-up mechanical testing by combining physiological relevance with a scalable throughput while providing an environment that reflects the mechanical properties of real human cardiac tissue."

Dr. Sonja Stoelzle-Feix, Director Scientific Affairs, Nanion Technologies, Germany

Nanion Europe

info@nanion.de

phone: +49 89 2190 95-0

www.nanion.de

Nanion USA

info@naniontech.com

phone: 1-888-9-NANION

www.naniontech.com

Nanion China

andy.di@nanion.cn

phone: +86 10 82 17 6386

www.nanion.cn

Nanion Japan

ohtsuki@nanion.jp

phone: +81 3 6457 8776

www.nanion.jp

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