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Approval section:

Position	Test facility manager:	QR:
Name		JWI
Signature		
Location, date	Neubiberg,	Neubiberg, 2022/04/07

Table of Contents

1 PURPOSE

Procedure for the evaluation of cellular response to a test medium.

This procedure describes the preparation, implementation and evaluation of a microphyiometric test using cellasys's IMOLA-IVD technology.

2 APPLICATION

6xIMOLA-IVD laboratories

3 QUALITY CONTROL

Document the performance of the tests with the form QC S001-002_cellasys#8.

4 OVERVIEW

The assay performs a negative control, blank and four replicates (see figure below). The assay includes a control that is fed with the reference medium (negative control) for 20 hours (green) and then with the reference medium supplemented with sodium lauryl sulfate (SDS) as positive control (dark grey). The blank is an empty control (without cells) to determine possible interactions between the sensors and the media. The assay enables the analysis of four replicated which are each treated with the test medium for a further six hours after six hours of reference medium(green). After another four hours with the reference medium (green), another treatment for four hours with the test medium takes place. Finally, media supplemented with SDS is applied as positive control (dark grey).
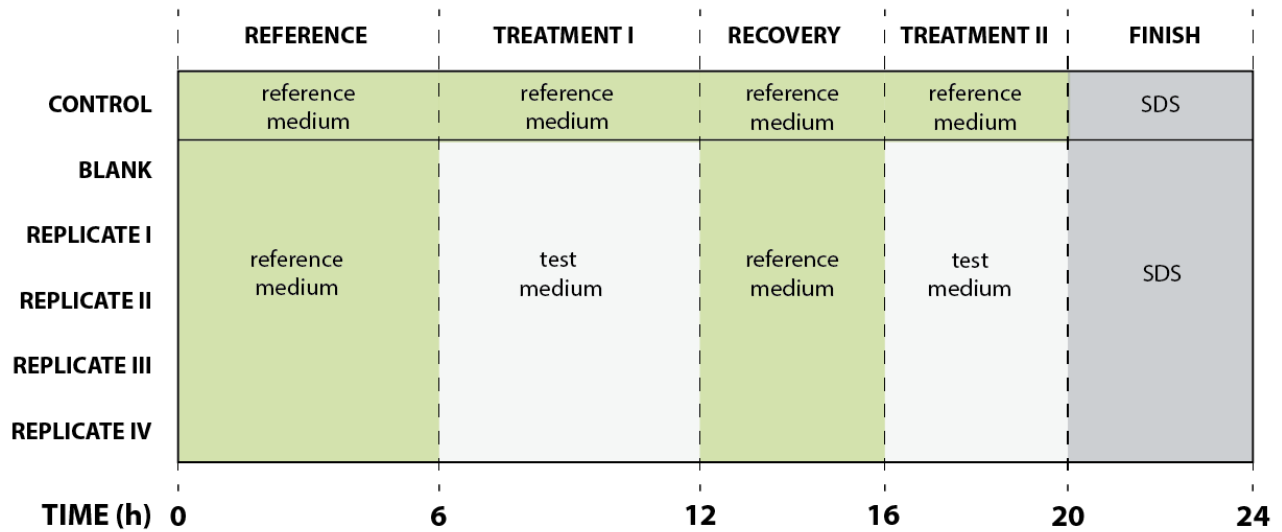
Figure 1: Setup of a cellasys #8 assay.

5 EQUIPMENT AND MATERIALS

5.1 TOOLS

Pos	Equipment
1	S1 laboratory
2	6xIMOLA-IVD
3	6x fluidic heads
4	12x O-Rings
5	18x 100 ml sterile bottles (GL45)
6	18x distribution caps with luer-steel needles
7	6x reference electrodes with barrels
8	6x flasks for sodium hypochlorite solution
9	6x flasks for ddH2O

5.2 CONSUMABLES & REAGENTS

Pos	Consumables
1	Cells under investigation (approx. 5x 100.000 in 300 µL reference medium)
2	6 x BioChips
3	Pipettes
4	Large petri dish (145x20)

Pos	Reagents
1	~ 2 % sodium hypochlorite solution
2	70 % denaturated ethanole solution
3	ddH2O
4	305 mL reference medium
5	325 mL test medium
6	Toxic substance (e.g. 3 ml 10% SDS solution)

5.3 SPECIAL SAFETY REQUIREMENTS

⚠	• put on gloves and a lab coat • use 70% ethanol for the item's desinfection

6 TIMETABLE

Day	Tasks
1 (afternoon)	1.1 Print and fill QC S001-002_cellasys_8 1.2 Setup 6xIMOLA-IVD 1.3 Sterilize 6xIMOLA-IVD 1.4 Prepare medium flasks 1.5 Setup 6xIMOLA-IVD with medium 1.6 1st Prefill 6xIMOLA-IVD with medium (set temperature) 1.7 Seed cells onto BioChips
2 (morning)	2.1 2nd Prefill 6xIMOLA-IVD with medium 2.2 Assemble BioChips with fluidic heads 2.3 Assemble BioChips into 6xIMOLA-IVD 2.4 Start experiment
3 (24 h's after start of experiment)	3.1 Stop experiment 3.2 Export data 3.3 Dismount 6xIMOLA-IVD

7 PROTOCOL

| Task	Description
1.1 QC S001-002_cellasys_8	- Print and fill QC S001-002_cellasys_8
1.2 Setup 6xIMOLA-IVD	- Start IMOLA_IVD (laptop, power supply, IMOLAs) - Close pump cartridges - Assemble and mount reference electrodes (6x)

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1.3 Sterilize 6xIMOLA-IVD	- Fill 6 flasks with sodium hypochlorite solution and put 4 (3) luer-steel connectors per IMOLA-IVD into it - Load application "cellasys #8 prefi-steri" - Press "START"and wait until time counts down from 86.000 seconds (it takes approx. 25 min)

1.4 Prepare medium flasks	- Prepare the following flasks with **reference medium (RM)**:
	
	   • **RM-**: 7x 40 mL
	   • **RM+**: 1x 25 mL with addition of 500 µL of 10% (w/v) SDS solution for positive control
	
	- Prepare the following flasks with the **treatment medium (TM)**:
	
	   • **TM-**: 5x 40mL
	   • **TM+**: 5x 25 mL with addition of 500 µL of 10% (w/v) SDS solution for positive control

1.5 Setup 6xIMOLA-IVD with medium
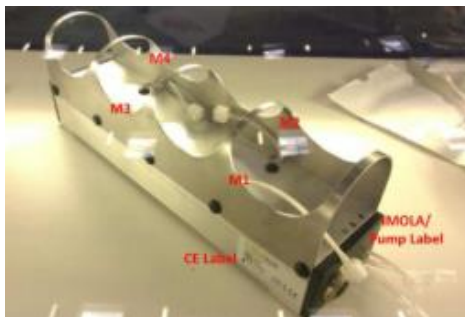 	
	- DALIA client: Press „STOP"
	
	- Load the **M2 position** (labelled at the fluidic modules) according to the following configuration:
	
	   • Control: **RM+**
	   • Blank, replicate I to IV: **TM+**
	
	- Load the **M3 position** according to the following configuration:
	
	   • Control: **RM-**
	   • Blank, replicate I to IV: **TM-**
	
	- Load the **M4 position** according to the following configuration:
	
	   • Control, blank, replicate I to IV: R**M-**

1.6 First Prefilling with medium	- DALiA client: Press „START" - Switch on 6xIMOLA-IVD incubator
1.7 Seed cells onto BioChips	- Seed cells in 300 µL medium (total volume with cells) onto BioChips for the control and replicate I to IV. - Add 300 µL growth medium only (no cells) to the blank - Place 6 BioChips in large petridish and place in CO2-incubator over night
2.1 Second Prefill 6xIMOLA-IVD with medium	- DALiA client: Press „STOP" - DALiA client: Press „START" - after approx. 25 min press „STOP"
2.2 Assemble BioChips with fluidic heads	- Make sure the luer connectors and clamps of the fluidic heads are open - Sterilize fluidic heads in sodium hypochlorite solution for 20 min and work in a sterile environment from this point - Flush 6 fluidic heads equipped with 6 O-rings and 6 O-Rings in ddH2O. - Get 6 BioChips from the incubator and gently press O-rings into BioChip housing (one O-ring per BioChip). Start with the BioChip for the blank (without cells) to prevent any cell takeover from the other BioChips seeded with cells. - Gently press fluidic heads into BioChips. - Close luer connectors of fluidic heads

2.3 Assemble BioChips into 6xIMOLA-IVD	- Connect BioChips with 6xIMOLA-IVD in the following configuration: • **Blank**: BioChip without cells • **Control and replicates**: 1,3,4,5,6: BioChips with cells - Connect luer connectors of the BioChips with the fluidic system.
2.4 Start experiment	- Load application cellasys #8 run - DALiA client: Press „START" - Let the experiment run for 24 h
3.1 Stop experiment / export data	- DALiA client: Press STOP - DALiA client → Database → Export format is start time of the experiment "yyyymmddhhmmss.exp"
3.2 Dismount 6xIMOLA-IVD	- Prepare 6x flasks with ddH2O - Dismount medium flasks with distribution caps (check remaining volume consistency); put Luer-steel tubes in flasks with ddH2O - Flush distribution caps with ddH2O and prepare for sterilization - Flush medium flasks with water and prepare for dishwasher - Disassemble BioChip / fluidic head / reference electrode setup - Separate BioChip / fluidic head / reference electrode / barrel - Flush BioChip, fluidic head and barrel with ddH2O

	- Store barrels of reference electrodes in 3 mol KCl - Run steri-prefi protocol (wait until countdown from 20 min) - Release cartridges of 6xIMOLA-IVDs pump - Copy .exp file for data processing and documentation - Shut down 6xIMOLA-IVD / PC - Empty waste flasks

8   DOCUMENTATION

Fill in QC S001-002_cellasys#8.

9   ARCHIVAL STORAGE

The original paper version of this document in its approved and released version is stored in the cellasys laboratory´s archive, located at:

cellasys laboratory

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10 RESPONSIBILITIES

Creation	Study Director
Approval	Testing Facility Manager
Archiving	Archiving Manager
Processing	Testing Personnel

11 REFERENCES
None.

12 ANNEXES
None.