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Project full title:

**Hepatic and Cardiac Toxicity Systems modelling**

Project acronym:

**HeCaTos**

Collaborative project

HEALTH.2013.1.3.-1:

Modelling toxic response in case studies for predictive human safety assessment

**FP7-HEALTH-2013-INNOVATION-1-602156-HeCaTos**

## **Deliverable Report D5.2: SOP for sample preparation**

Work package 5

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**Maastricht University (UM)**

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Dissemination Level		
<b>PU</b>	Public	X
<b>PP</b>	Restricted to other programme participants (including the Commission Services)	
<b>RE</b>	Restricted to a group specified by the consortium (including the Commission Services)	
<b>CO</b>	Confidential, only for members of the consortium (including the Commission Services)	

## Contributions to deliverable - Internal review procedure

Deliverable produced by:	Date:
Adrian Roth - Partner ROCHE	November 2014
Ramona Nudischer - Partner ROCHE	November 2014
Adrian Roth - Partner ROCHE	November 2014
Deliverable internally reviewed by:	Date:
Jos Kleinjans - Partner UM	November 2014

## Contents

Publishable Summary.....	3
Objectives .....	3
Introduction .....	3
Results.....	3
Difficulties .....	5

## PUBLISHABLE SUMMARY

WP5 is taking care of drug treatment experiments. At the end of each experiment, Microtissues are harvested, frozen and stored. Frozen samples then are shipped to collaboration partners taking care of downstream analysis as listed below. For some of the readouts, experiments are carried out at the site of the analytical lab and in such a case fresh microtissue plates are shipped.

## OBJECTIVES

The objective was to set up a workflow which allows generation of samples and subsequent shipment to analytical groups.

## INTRODUCTION

To guarantee highest quality results, a smooth workflow from drug treatment to harvesting and analysis of samples is key. Within this consortium which consist of *in vitro* labs, analytical labs and data analysis labs, the interfaces which handle samples from one place to the other are key and this is what we have been working on.

## RESULTS

The preparation of samples has been optimized to meet the needs for the individual downstream analysis:

### 1.) RNA sampling:

Total RNA isolation isolated from Insphero human liver microtissues using the Maxwell® 16 LEV simply RNA Tissue Kit (semi-automated method).

#### Microtissues:

3D InSight™ Human liver microtissues

3D InSight™ Human cardiac microtissues

#### Materials needed:

3D InSight™ Human Liver Microtissues – Insphero - ~200um in diameter

Maxwell® 16 Instrument (AS2000)

Maxwell® 16 LEV simplyRNA Tissue Kit (AS1280)

Quantus™ Fluorometer (E6150)

QuantiFluor® RNA system (E3310)

Optional: Handheld Tissue homogenizer (Fisherbrand™ Pellet Pestle™ Cordless Motor)

#### Protocol :

Microtissue preparation:

1. Collect 30 ul of supernatant for metabolomics into a non-steril, blank 96well plate with lid ( → see 4.) metabolomic);
2. Pool defined number of microtissues by using a 1000 ml Pipette and aspire remaining liquid in the plate (including microtissue) in excess. Collect microtissues in a 1.5ml tube;
3. Wash samples with 50µl of 1x PBS;

4. Continue directly to RNA isolation.

RNA isolation - Maxwell® 16 LEV simplyRNA Tissue Kit:

1. Prepare the Maxwell® 16 instrument, reagents, and cartridges as described in [TM351](#);
2. Add 200µl of chilled 1-Thioglycerol/Homogenization Solution to each sample;
3. Vortex samples for 10 seconds and pipet up and down 10 times to lyse microtissues. Optional: For more complete lysis: use a handheld homogenizer for 5-10 seconds;
4. Add 200µl of Lysis Buffer to each of the lysed microtissues. Vortex the lysates vigorously for 5 seconds to mix;
5. Transfer all ~400µl of lysate to well 1 of the Maxwell® 16 LEV Cartridge;
6. Add 5µl of DNase I solution to well 4 of each cartridge;
7. Add 50µl of water to the elution tubes and place in cartridge rack;
8. On the Maxwell® 16 instrument, select “RNA”, followed by “simplyRNA”, then select “simplyRNA” once more on the Menu screen;
9. After the method is complete, place eluates on ice;
10. Determine RNA concentrations and yields using the QuantiFluor® RNA system with the Quantus™ Fluorometer. For more details see [TM396](#);
11. Store sample at -80°C, Ship to UM, Maastricht.

Labeling of mRNA samples: Sample IDs will be specified in an overview table which is send via email to the consortium partner:

Sample ID	Date	Compound	Incubation time with respective concentration	dosis
#0001M	071114	APAP	1 hours 1.3uM	Therapeutic [1.3uM]
#0002M	071114	APAP	1 hours 1.3uM 2 hours 0.65 uM	Therapeutic [1.3uM]

## 2.) DNA sampling:

1. Pool defined number of microtissues (collect supernatant for metabolomics! → see 4.) metabolomic);
2. Wash samples with HBSS;
3. add 500ul DNA-specific lysis buffer (EDTA, SDS, Tris, H2O);
4. Store at -20°C and ship to MPIMG, Berlin.

Labeling of DNA samples: Sample IDs will be specified in an overview table which is send via email to the consortium partner:

Sample ID	Date	Compound	Incubation time with respective concentration	dosis
#0001DNA	071114	APAP	1 hours 1.3uM	Therapeutic [1.3uM]
#0002DNA	071114	APAP	1 hours 1.3uM 2 hours 0.65 uM	Therapeutic [1.3uM]

## 3.) Proteomics:

1. Pool defined number of microtissues (collect supernatant for metabolomics! → see 4.) metabolomic);
2. Wash samples with 1x PBS;
3. Flush in liquid nitrogen;
4. Store at -80°C and ship to FHGZ, Zürich.

Labeling of Proteomic samples: Sample IDs will be specified in an overview table which is send via email to the consortium partner:

Sample ID	Date	Compound	Incubation time with respective concentration	dosis
#0001P	071114	APAP	1 hours 1.3uM	Therapeutic [1.3uM]
#0002P	071114	APAP	1 hours 1.3uM 2 hours 0.65 uM	Therapeutic [1.3uM]

#### 4.) Metabolomics:

1. Collect supernatants from RNA sampling, DNA sampling, and Proteomics in non-steril, blank 96well plate with lid;
2. seal plate, store at -80°C, and ship to Imperial College London.

Labeling of Metabolomics samples: Sample IDs will be specified in an overview table which is send via email to the consortium partner:

Sample ID	Date	Compound	Incubation time with respective concentration	dosis
#0001M	071114	APAP	1 hours 1.3uM	Therapeutic [1.3uM]
#0002M	071114	APAP	1 hours 1.3uM 2 hours 0.65 uM	Therapeutic [1.3uM]

#### 5.) Functional Assays (Oxygen consumption, Extracellular acidification, Intracellular Oxygen, Reactive Oxygen Species and ATP):

Fresh Microtissues are shipped to Luxcel for compound testing.

#### DIFFICULTIES

None.