

## **<sup>13</sup>C Tracer Analysis and Metabolomics in 3D Cultured Cancer Cells**

Created on: 04-08-2020 - Last modified on: 31-08-2023

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## **SCOPE OF THE METHOD**

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Specify the type of cells/tissues/organs</b>	human cancer cell lines

## **DESCRIPTION**

### **Method keywords**

3D Cell culture  
Cancer cells  
Metabolism  
Metabolic quenching  
spheroids  
metabolomics

### **Scientific area keywords**

Cancer metabolism  
<sup>13</sup>C tracer analysis  
3D  
spheroids  
extracellular matrix  
cancer  
metastasis

### **Method description**

Metabolomics and  $^{13}\text{C}$  tracer analysis are state-of-the-art techniques that allow determining the concentration of metabolites and the activity of metabolic pathways, respectively. Three dimensional (3D) cultures of cancer cells constitute an enriched *in vitro* environment that can be used to assay anchorage-independent growth, spheroid formation, and extracellular matrix production by (cancer) cells. Here, we describe how to perform metabolomics and  $^{13}\text{C}$  tracer analysis in 3D cultures of cancer cells. Intracellular metabolites are extracted from these quenched cells, and  $^{13}\text{C}$ -label incorporation patterns and metabolite levels are determined via MS-based analysis.

### **Lab equipment**

- Humidified temperature and  $\text{CO}_2$ -controlled cell culture incubator ;
- Vacuum aspirator ;
- Biological safety cabinet ;
- Cell counter ;
- Thermometer ( $-40^\circ\text{C}$  to  $0^\circ\text{C}$ ) ;
- Chemical fume hood ;
- Tissue Lyser ;
- Heating block ;
- Centrifuge fitting 15 and 50 mL tubes ;
- Vortex ;
- Refrigerated centrifugal vacuum concentrator ;
- Mass Spectrometer with gas or liquid chromatographic technique.

### **Method status**

Published in peer reviewed journal

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

A key feature of the method lies in the possibility to study metabolism in cells growing in an anchorage-independent manner which might be more representative of the physiological status of metastatic cancer cells. This allows us to recapitulate some cancer phenotypes of disseminated cancer cells during the metastatic process.

In addition, the described quenching process enables to rapidly stop the metabolism of cancer cell spheroids as well as to measure the concentration and labeling patterns of metabolites in a reliable manner.

### **Challenges**

To obtain accurate metabolic measurements you need to have an adequate amount of cells to extract. When growing cells in this system, the number of spheroids is limited per well to avoid a group of cells to grow as a single spheroid (which will not recapitulate the metabolism of colonizing cells). Therefore, it is necessary to optimize the number of wells to pool together according to cell type and condition to test to achieve the proper amount of cell extracts.

Metabolic quenching of spheroids that grow in suspension is a more time-consuming protocol compared to the quenching of attached cells. Since metabolism is a rapidly adapting process, it is critical to work as fast as possible and maintain low temperature during the process to minimize the chance of perturbations in cellular metabolism.

### **Modifications**

This protocol can be adapted for metabolic measurements of alternative systems of cells growing in suspension.

## Future & Other applications

This protocol can be adapted for metabolic measurements of alternative systems of cells growing in suspension.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### Links

[13C Tracer Analysis and Metabolomics in 3D Cultured Cancer Cells](#)

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