

# Assessing the impact of the nutrient microenvironment on the metabolism of effector CD8<sup>+</sup> T cells

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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>Species from which cells/tissues/organs are derived</b>	Mice

Type of cells/tissues/organs	Spleen
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## DESCRIPTION

### Method keywords

Nutrient

Microenvironment

Metabolism

CD8+ T cells

Physiological blood-like medium

cell culture

Metabolic flux

Metabolic quenching

Custom media formulations

### Scientific area keywords

Immunometabolism

CD8+ T cells

$^{13}\text{C}$  tracer analysis

Nutrient microenvironment

### Method description

The method is an approach to systematically study the impact of the nutrient microenvironment on the metabolism of effector CD8+ T cells, based on performing stable  $^{13}\text{C}$  isotope labeling measurements on *in vitro*-differentiated murine effector CD8+ T cells. Naive CD8+ T cells are isolated from mouse spleens, further activated and differentiated into an effector state *in vitro*. Effector CD8+ T cells are then cultured under different medium conditions, in the presence of  $^{13}\text{C}$  isotope tracers. Intracellular metabolites are extracted from these cells, and  $^{13}\text{C}$ -label incorporation patterns and metabolite levels

are determined via MS-based analysis. Coupling this information with growth rates, metabolite uptake and secretion rates, and an appropriate metabolic flux model, you will be able to assess the impact of the different nutrient conditions on the metabolism of effector CD8<sup>+</sup> T cells.

## **Lab equipment**

Chemical fume hood ;

Biological safety cabinet ;

pH meter ;

Centrifuge fitting 15 and 50 mL tubes ;

Humidified, temperature and CO<sub>2</sub>-controlled cell culture incubator ;

Vacuum aspirator ;

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 cell culture medium formulation, or Blood-Like Medium (BLM). The latter has been adapted from the literature to match the concentrations found in human plasma for a variety of nutrients, in order to provide results more representative of physiological conditions. In addition, the described BLM formulation has the further advantage of being easily customizable, allowing to pull out a variety of individual nutrients (in particular, several non-essential amino acids, glucose, and pyruvate) with minimum work. This enables replacing these nutrients with <sup>13</sup>C-labeled analogs of choice, or to investigate the impact of the full or partial depletion of any (or a combination) of them on the metabolism of effector CD8<sup>+</sup> T cells. You reduce the amount of mice used by testing different conditions in culture on T-cells from one mouse.

### **Challenges**

This technique still doesn't fully recapitulate the *in vivo* immune response although it is already a step in the good direction. For this method, you will still need to collect a mouse spleen. T-cells are small cells which means you will need an adequate number of cells to get accurate metabolite measurements. T-cells are cultured in suspension. Metabolism is a rapidly adapting process. Therefore, it is critical to work as fast as possible during the metabolic quenching. Metabolic quenching of cells in suspension takes longer than quenching attached cells. This will increase the chance of perturbations to cellular metabolism.

## Future & Other applications

The easily customizable blood-like medium can also provide results more representative of physiological conditions in other applications.

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### Links

[Assessing the Impact of the Nutrient Microenvironment on the Metabolism of Effe...](#)

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