Common Media Supplements for XF Assays

Technical Overview

Introduction

When measuring cellular metabolism, it is critical to include all of the necessary substrates for the specific XF assay. This document outlines recommendations for assay media supplements and their function within an XF assay. Additional modifications may be made depending on experimental conditions. Refer to the specific XF assay user guide or protocol for assay supplement information and concentration.

Intact Cells

Supplement	Which XF assay?	Why is it needed in my XF assay?
Glucose	Agilent Seahorse XF Cell Mito Stress Test, FAO Assay	Carbon source that drives glycolysis, pyruvate production and mitochondrial respiration
Pyruvate	Agilent Seahorse XF Cell Mito Stress Test	Carbon source that supports the TCA cycle and fuels mitochondrial respiration
Glutamine	Agilent Seahorse XF Cell Mito Stress Test Agilent Seahorse XF Glycolysis Stress Test	Carbon source that supports the TCA cycle and fuels mitochondrial respiration
Palmitate-BSA	FAO assay	Long chain fatty acid; fuels mitochondrial respiration
Carnitine	FAO assay	Required for fatty acid transport to the mitochondria

Permeabilized Cells or Isolated Mitochondria

Supplement	Which XF Assay?	Why is it needed in my XF Assay?
ADP	Agilent Seahorse XF PMP Assay Isolated mitochondria	Substrate for Complex V (ATP synthase); Precursor to ATP; Stimulates mitochondrial respiration
Pyruvate/Malate	Agilent Seahorse XF PMP Assay Isolated mitochondria	NADH-linked (Complex I) substrates
Glutamate/Malate	Agilent Seahorse XF PMP Assay Isolated mitochondria	NADH-linked (Complex I) substrates
Succinate/Rotenone*	Agilent Seahorse XF PMP Assay Isolated mitochondria	Q-linked (Complex II or III) substrates
Palmitoyl carnitine/Malate	Agilent Seahorse XF PMP Assay Isolated mitochondria	NADH-linked (Complex I) substrates and $FADH_2$-linked (Complex II) substrates
Ascorbate/TMPD	Agilent Seahorse XF PMP Assay Isolated mitochondria	Cytochrome *c* oxidase-linked (Complex IV) substrates

*Rotenone, when used in combination with succinate in permeabilized cells or isolated mitochondria, functions as media supplement.

For More Information

Contact Agilent Seahorse Technical Support at: support@seahorsebio.com

www.agilent.com/chem

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