Characterizing Your Cells

Using OCR Values to Determine Optimal Seeding Density

Technical Overview

Introduction

There are two critical elements to any XF assay: using supplemented assay medium (as directed by the experimental conditions) and identifying an optimal cell seeding density[1]. This document outlines recommendations for cell characterization of seeding density using basal oxygen consumption rate (OCR) values.

Visual assessment is used first to approximate optimal cell density: cells should be at 50–90 % confluence, evenly distributed within each individual well, and across each well of the plate. Basal OCR values can confirm optimal seeding density, as well as ensure data accuracy and precision.

Cell Seeding Assessment

The basal parameter is used to assess seeding density, as the cells are offered an unlimited supply of substrate and have not yet been treated with drugs or inhibitors. Follow the desired XF assay instructions outlined in the specific user guide or protocol to ensure data quality. Use the indicated measurement points for basal OCR readings.

Assay	Measurement point
Agilent Seahorse XF Cell Mito Stress Test	Use the last measurement prior to the oligomycin injection.
Agilent Seahorse XF Glycolysis Stress Test	Use the last measurement in the presence of glucose prior to the oligomycin injection.
Agilent Seahorse XFp Cell Energy Phenotype Test	Use the last measurement prior to the injection.

Data Analysis

Agilent Seahorse recommends the following basal OCR ranges:

Instrument	Basal OCR range (pmol/min)
Agilent Seahorse XFe24, XF24	50–400
Agilent Seahorse XFe96, XF96, XFp	20–160

When deciding between two seeding densities that have basal OCR values within the indicated ranges, use the following basal extracellular acidification rate (ECAR) ranges at the same measurement points selected for the OCR values:

Instrument	Basal ECAR range (mpH/min)
Agilent Seahorse XFe24, XF24	20–120
Agilent Seahorse XFe96, XF96, XFp	10–90

1. For further details on seeding optimal cell densities, refer to the Basic Procedures

For More Information

Contact Agilent Seahorse Technical Support at: seahorse.support@agilent.com

www.agilent.com/chem/discoverXF

Published in the USA, October 26, 2017
5991-7994EN

Agilent Technologies