Technical Overview

Cell Analysis

Agilent

Trusted Answers

Characterizing Your Cells

Using confluence and OCR values to determine optimal seeding density

Introduction

There are two critical elements to any Agilent Seahorse XF Assay: identifying an optimal seeding density and selecting the assay medium composition compatible with the XF system. This document outlines recommendations for cell characterization of seeding density using visual assessment and oxygen consumption rate (OCR) values.

Visual assessment

Cells should be at 75 to 90% confluence in a single monolayer, evenly distributed within each individual well, and across each well of the plate. Ensure cells are not overconfluent or clumping.

OCR assessment

Basal OCR measurement is used to assess seeding density. Use the last measurement prior to any injections.

Agilent recommends the basal OCR ranges in Table 1. These recommended basal OCR ranges allow for reliable detection of changes after compound addition. Note, these are not the full working OCR ranges for Agilent Seahorse XF analyzers.

Table 1. List of OCR ranges.

Instrument	Microplate	Basal OCR Range (pmol/min)
Seahorse XF Flex, XFe24	XF24 V7	50–400
Seahorse XF Pro, XFe96, HS Mini, XFp	XFe96/XF Pro, XFp	20–160
Seahorse XF HS Mini	HS Miniplate	7–55

When deciding between two seeding densities that are 75 to 90% confluent and within the indicated basal OCR ranges, use the basal extracellular acidification rate (ECAR) recommendation in Table 2.

Table 2. List of ECAR ranges.

Instrument	Microplate	Basal ECAR Range (mPH/min)
Seahorse XF Flex, XFe24	XF24 V7	20–120
Seahorse XF Pro, XFe96, HS Mini, XFp	XFe96/XF Pro, XFp	10–90
Seahorse XF HS Mini	HS Miniplate	10–90

For more information contact Agilent Seahorse Technical Support at **cellanalysis.support@agilent.com**

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DE-009526

Published in the USA, September 8, 2025
5991-7994EN