

Errata Notice

This document contains references to BioTek. Please note that BioTek is now Agilent. For more information, go to www.agilent.com/lifesciences/biotek



BioTek Resources for Agilent MitoXpress® Intra Intracellular Oxygen Assay

Introduction

The Agilent MitoXpress® Intra Intracellular Oxygen Assay allows real-time measurement of intracellular oxygen levels of whole cells in 2D or 3D cell cultures. BioTek offers a dedicated filter cube assembly, pre-programmed Gen5™ protocol, and user tutorial specifically optimized for use with the lifetime signal acquisition of the assay on BioTek's Cytation™ 1, Cytation 5, Synergy™ H1, or Synergy Neo2 instruments equipped with TRF capability.



Agilent MitoXpress Intra Intracellular Oxygen Assay User Tutorial:

<https://www.biotek.com/applications/cell-based-assays.html>

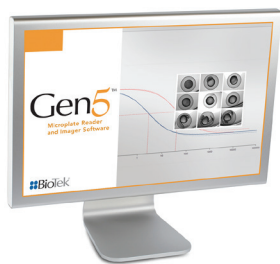


BioTek Filter Cube P/N 8040587 or P/N 8040594 (Cytations, Synergy H1)

BioTek Filter Cube P/N 1035123 (Synergy Neo2)

MitoXpress.XML (Synergy Neo2)

<https://www.biotek.com/resources/sample-files/mitoxpress/>



Gen5 Data Analysis Protocol: AgilentIntraO2.prt

<https://www.biotek.com/products/software-robotics-software/gen5-microplate-reader-and-imager-software/software/>

Note: The User Tutorial and Data Analysis Protocols are compatible with Gen5 v3.04 software or higher. Existing Gen5 users can upgrade their v3.0x software for free at: <https://btresource.force.com/CRC/s/article/Gen5-3-05>

Key Words:

Cellular Metabolism
Intracellular Oxygen Assay
Cellular Respiration
Cellular Oxygenation
Oxidative Stress

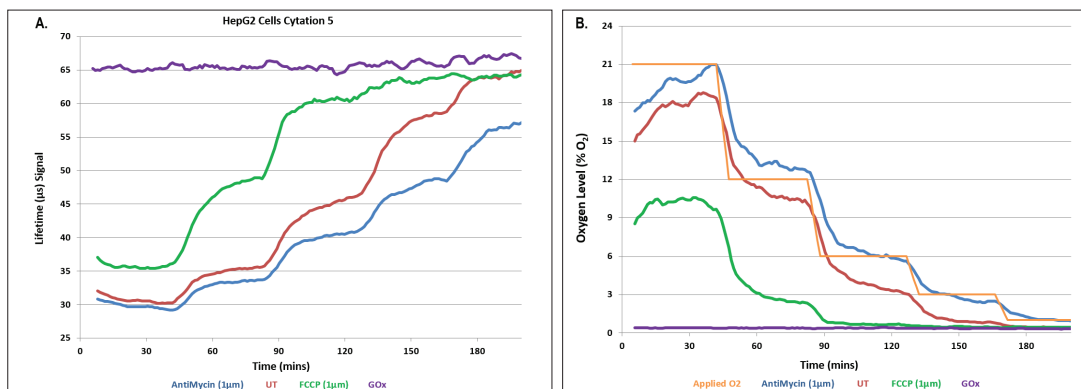


Figure 1. Example Data (Cytation 5): (A.) Lifetime detection of intracellular oxygenation in HepG2 cells during stepped decreases in oxygen within the read chamber. (B.) Intracellular O₂ concentration can be calculated from a first order exponential fit on the lifetime values and is shown compared to the applied instrument oxygen levels (orange).

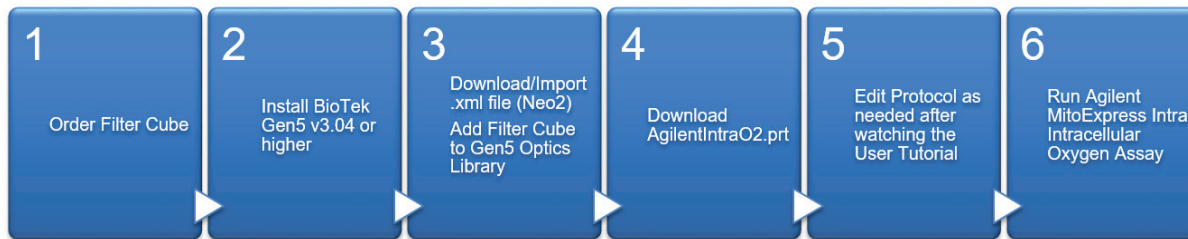


Figure 2. Top level implementation steps for the Agilent MitoXpress Intra Intracellular Oxygen Assay resources for Time-Resolved Fluorescence (TRF) equipped BioTek Cytation™ 1, Cytation™ 5, Synergy™ H1, or Synergy™ Neo 2 instruments.

Procedure

| Description | Comments |
|--|----------|
| Comment Edit incubation parameters as required | |
| Temperature: Setpoint 37 °C | |
| Comment Edit kinetic loop interval as required | |
| Comment The kinetic interval below is based on reading wells B2..... | |
| Start Kinetic [Run 3:45:00, Interval 0:01:00] | |
| Read: 30_30 (F) 380/20,645/15 | B2..G11 |
| Read: 70_30 (F) 380/20,645/15 | B2..G11 |
| End Kinetic | |

Plate Layout

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|-----|---------|------|------|------|------|------|-------|-------|-------|----|
| A | | | | | | | | | | | | |
| B | | BLK | NoCells | FCCP | SMP1 | SMP3 | SMP5 | SMP7 | SMP9 | SMP11 | SMP13 | |
| C | | BLK | NoCells | FCCP | SMP1 | SMP3 | SMP5 | SMP7 | SMP9 | SMP11 | SMP13 | |
| D | | BLK | NoCells | FCCP | SMP1 | SMP3 | SMP5 | SMP7 | SMP9 | SMP11 | SMP13 | |
| E | | GOX | AA | UT | SMP2 | SMP4 | SMP6 | SMP8 | SMP10 | SMP12 | SMP14 | |
| F | | GOX | AA | UT | SMP2 | SMP4 | SMP6 | SMP8 | SMP10 | SMP12 | SMP14 | |
| G | | GOX | AA | UT | SMP2 | SMP4 | SMP6 | SMP8 | SMP10 | SMP12 | SMP14 | |
| H | | | | | | | | | | | | |

Data Reduction

| Description | Data Out | Comments |
|----------------------|---------------------------|-------------------|
| Blank Transformation | Blank 30_30:380/20,645... | Blank Subtraction |
| Blank Transformation | Blank 70_30:380/20,645... | Blank Subtraction |
| Transformation | Lifetime | |
| Transformation | MeanLifetime | |
| Transformation | PercentIntraO2 | |

Figure 3. The AgilentIntraO2.prt procedure, plate layout, and data reduction steps as shown from within Gen5™ software.