

## BioTek Resources for Agilent MitoXpress® Intra Intracellular Oxygen Assay



### Key Words:

Cellular Metabolism  
Intracellular Oxygen Assay  
Cellular Respiration  
Cellular Oxygenation  
Oxidative Stress

### 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 the BioTek 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>

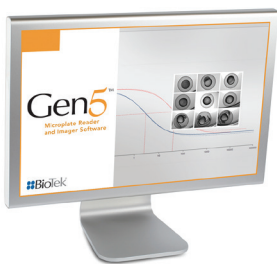


### BioTek Filter Cube P/N 8040587 FLTR BLK MITOXPRESS ASBY (Cytation, Synergy H1)

### BioTek Filter Cube P/N 1035123 CUBE 123 MITOXPRESS ASBY (Synergy Neo2)

MitoXpress.XML (Synergy Neo2)

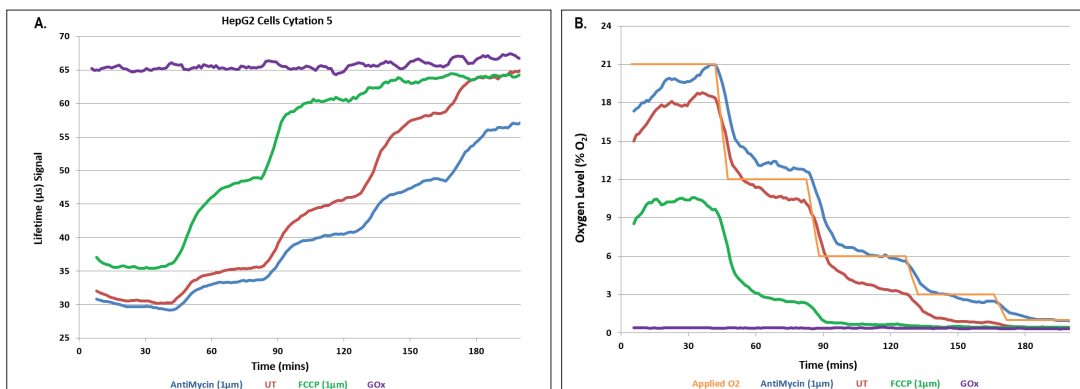
<https://www.biotek.com/resources/sample-files/mitoxpress/>



### 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>



**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 O<sub>2</sub> 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).

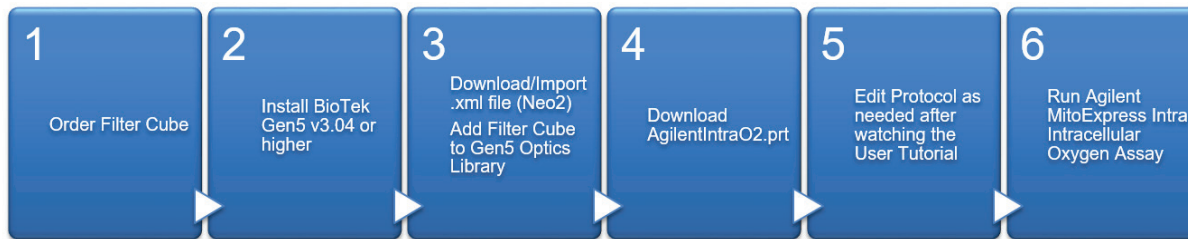


Figure 2. Top level implementation steps for the Agilent MitoXpress Intra Intracellular Oxygen Assay resources for Time-Resolved Fluorescence (TRF) equipped BioTek Cytation™ 1, Cytation™ 5, Synergy™ H1, or Synergy™ Neo 2 instruments.

**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.