

Oct 17, 2019 Version 2

## Microplate Reader Workflow V.2

DOI

[dx.doi.org/10.17504/protocols.io.8bqhsmw](https://dx.doi.org/10.17504/protocols.io.8bqhsmw)

NUS iGEM<sup>1</sup>

<sup>1</sup>National University of Singapore



**NUS iGEM**

National University of Singapore

---

OPEN  ACCESS



DOI: [dx.doi.org/10.17504/protocols.io.8bqhsmw](https://dx.doi.org/10.17504/protocols.io.8bqhsmw)

**Protocol Citation:** NUS iGEM 2019. Microplate Reader Workflow. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.8bqhsmw>

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

**We use this protocol and it's working**

**Created:** October 17, 2019

**Last Modified:** September 12, 2023

**Protocol Integer ID:** 28752



1 Refresh overnight cell culture in LB media at 37 °C

### Protocol



NAME

## Preparation of LB Media

CREATED BY  
NUS iGEM

**PREVIEW**

---

1.1 Weigh 25 g of Luria Broth Base powder.



Luria Broth Base (Miller's LB Broth Base)&trade;, powder **Thermo**  
**Fisher Catalog #12795027**

1.2 Add the powder into 1 L of water.



Water refers to sterilized deionized water

1.3 Autoclave entire bottle of LB media.



2 Measure OD<sub>600</sub> of cell culture for desired OD value

3 Load 200 µL of cell samples into three wells of a 96-well plate (triplicates)

4 Induce appropriate volumes of chemical inducers (not exceeding 6 µL ) in each well

5 Include LB media as blanks

## 6 Load the plate (with lid) into microplate reader

Equipment	
Cytation 5	NAME
Automated microscope; Multi-mode plate reader	TYPE
BioTek	BRAND
N.A.	SKU
<a href="https://www.biotek.com/products/cytation.html">https://www.biotek.com/products/cytation.html</a>	LINK
 	

## 7 Start protocol to run continuous measurement overnight



## Note

### **OD<sub>600</sub> (absorbance) protocol:**

Wavelength: 600nm

Measurement interval time: 8min

Run time: 18h

### **GFP (fluorescence) protocol:**

Excitation: 485 +/- 10

Emission: 528 +/- 10

Gain: Extended

### **RFP (fluorescence) protocol:**

Excitation: 535 +/- 20

Emission: 600 +/- 20

Gain: Extended

### **Luminescence protocol:**

Gain: 118

Integration time: 1s

### **OD<sub>600</sub> + GFP protocol script:**

Measurement interval time: 8min

Run time: 18h

### **OD<sub>600</sub> + luminescence protocol script:**

Measurement interval time: 20min

Run time: 20h