

RIO-UFRJ-BRAZIL	<b>Protocol</b>	Page 1 of 2
Created by Lucas Santiago		
Reviewed by Monica Lomeli e Claudio Masuda		
Subject: Glucose Dosage		

**Glucose Dosage:**

Standard curve

well	Glucose 1mg/ml	H <sub>2</sub> O	Glucos (reagente1)	DO 510 nm
1	0µl	20 µl	250 ul	
2	0	20	'	
3	2	18	'	
4	4	16	'	
5	6	14	'	
6	8	12	'	
7	10	10	'	
	YPD 1/20		'	
8	2	18	,	
9	4	16	,	
10	6	14	,	

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#### Culture samples

well	Amostra diluída			
	1/20			
1	10ul	10		
2	20ul	0		

- Dilute the glucose 100 mg/mL to 1 mg/mL;
- Dilute the samples and medium without cells to 1:100;
- Make a serial dilution of glucose for to standard curve;
- Make a serial dilution of medium to determine the initial glucose concentration;
- Use one well to pipette 20 uL of sample and other to dilute the sample 1:2;
- Add 250 uL of glucose reaction;
- Use water as the blanco;
- Incubate for 10 minutes at 37°C;
- Read at 505 nm;
- Diminish the absorbances to the blanco;
- Make the standard curve using the absorbance with the known glucose concentration;
- Use the line equation generated by standard curve to calculate initial concentration of glucose in medium without cells;
- Use the same equation to calculate the concentration of samples;

Consider the dilutions made in the equation.