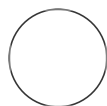# 🌐 Glucose Concentration assay (Hexokinase/G6PDH method)

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We use this protocol and it's working

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DISCLAIMER

ABSTRACT

The assay I describe measures glucose concentration in a sample. The principle of the assay is to consume glucose from the sample with hexokinase and excess ATP in the buffer, then to oxidize the resulting glucose-6-phosphate and simultaneously generate NADPH from excess NADP+ in the buffer. NADPH can be observed on a microplate reader because it absorbs light at 340 nm. I adapted this assay for use in a microplate reader from "A flexible system of enzymatic analysis" by Oliver Lowry and Janet Passoneau. It is reliable, inexpensive, and diagnostically useful for calculating cell or media glucose consumption rates.

IMAGE ATTRIBUTION

Image made using

- Advice corresponding to individual steps is placed in the step's "notes" section
- There are alternatives to some of the materials used herein. If you prefer to measure fluorescence, your plate does not need a clear bottom. Also, cheaper alternatives to chemicals such as NADP+ exist and are sold by other companies. The chemistry of these products is unlikely to change the assay performance.

MATERIALS

Consumables

- 96-well microplate (Corning, 3603) - black walled for fluorescence, clear bottom for absorbance
- 20 and 200 uL pipette tips
- (optional) Reagent reservoir

Reagents

- D-glucose (for standards, G7528)
- Tris Base, pH8 (from T1503)
- $MgCl_2$ (Fisher, M33-500)
- $NADP^+$ (N0505)
- ATP (Sigma, A2383)
- Hexokinase (Sigma, H4502)
- glucose-6-phosphate dehydrogenase (G6378)
- dH_2O

Equipment

- Pipette (200 and 20 uL capacity should suffice for this assay)
- (optional) multichannel pipette (200uL capacity)
- Plate reader that can determine absorbance at 340 nm or fluorescence (ex/em of 340/460) (for us, a BioTek Synergy 4)
- A balance, accurate to ~5 mg

BEFORE START INSTRUCTIONS

- Read the instructions! That's it I think...

Generate glucose standards

1   Make a D-glucose stock solution (for me, normally 500 mM), and dilute to make standards. My typical standards are 0-10 mM (10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0 mM). The composition of standards may of course be adapted to your needs.

Note:
- The range of the standard curve must exceed the range of the samples you will use.

Otherwise the data may not be as interpretable as it appears.
- If you plan to increase the concentration of the samples/standards you use, I recommend lowering the volume of standards and samples in the assay.
- As you decrease sample/standard concentration, I recommend increasing the volume of standards and samples in the assay.

Prepare assay buffer

2   For 50 mL of assay buffer (enough for ~2.5 96 well plates, can be scaled). I usually add the following components in order

Stock solutions	Volume used
1M Tris base, pH8	2.5 mL
1M MgCl$_2$	50 uL
dH$_2$O	47.45 mL
Add solid:	
NADP+	19.7 mg
ATP	14.6 mg
hexokinase	10 U
glucose-6-phosphate dehydrogenase	4 U

Once all components are in the buffer, mix by inversion until all components are dissolved and uniformly distributed.

The final concentration of components: 50 mM Tris, 1 mM MgCl2, 500 μM NADP+, 500 μM ATP, 0.2 U/mL Hexokinase, 0.08 U/mL glucose-6-phosphate dehydrogenase, pH 8.1.

Note:
- Now is also a good time to pre-warm your spectrophotometer to 37 degrees C. If your spectrophotometer does not have a warming function, that is ok, but as a consequence reaction times may take longer to reach completion.
- Once made, the assay buffer works for at least a week. Over time the buffer will gain a yellow hue, which should result from auto-reduction of NADP$^+$ to NADPH. If there is a visible hue, it would be best not to use the buffer, and make a new stock instead.

Add samples and standards to plate

3   Add samples and standards to the microplate. 2-10 uL for samples/standard.

Note:
- Samples and standards should be added at least in duplicate
- The volume of samples and standards is best kept consistant, as this simplifies the downstream calculations

- 2-10 uL at ~1-10 mM glucose should yield good data.

4 Add 195 uL assay buffer to the plate.

Note:
- Ensure that the addition of assay buffer follows the addition of standards and samples.
- The addition of a larger volume helps to mix the samples with the buffer.

Read plate

5 Insert plate with samples, standards, and assay buffer into the pre-warmed spectrophotometer *(if it has not been pre-warmed or cannot warm up, the assay can still proceed but may do so more slowly)*.

6 Read absorbance at 340 nm or fluorescence (ex:340, em:460) to determine NADPH levels in samples and standards. Read multiple times over ~60-90 minutes, or until the reaction has stopped. The reaction stops when absorbance or fluorescence no longer increases over time.

Note:
- Absorbance values approximately >2 are normally outside the dynamic range of quantitation that you should trust. You may attempt to salvage the sample by diluting it in assay buffer, re-reading, and compensating for the dilution factor in your calculations. This may not work, in which case the next time you perform the assay, attempt a sample dilution series to determine the concentrations that lie within your standard curve and within the range of accurate quantitation by your spectrophotometer. Otherwise Determine whether

Analyze data

7 Once absorbance at 340 nm or fluorescence has reached a steady-state, export data into a spreadsheet. The standard curve should fit a linear function well ($R^2 \geq 0.99$). If this is the case, use this linear function to determine sample concentration.

Note:
- Some users have reported that their data does not fit a linear function.
- An example spreadsheet is attached 📎 glucose_assay_example.xlsx