ab65333 – Glucose Assay kit (Colorimetric/Fluorometric)

For the rapid, sensitive and accurate measurement of Glucose in various samples.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit: www.abcam.com/ab65333
(use www.abcam.cn/ab65333 for China, or www.abcam.co.jp/ab65333 for Japan)

This product is for research use only and is not intended for diagnostic use

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

- Aliquot components in working volumes before storing at the recommended temperature.

Materials Supplied:

Item	Quantity	Storage temperature (before preparation)	Storage temperature (after preparation)
Assay Buffer II/Glucose Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/Glucose Probe (in DMSO)	200 µL	-20°C	-20°C
Development Enzyme Mix II/Glucose Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
Glucose Standard/100 nmol/µL Glucose Standard	100 µL	-20°C	-20°C

Materials Required, Not Supplied

These materials are not included, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- MilliQ water or other type of double distilled water (ddH2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom (for colorimetric assay) / 96 well plate with clear flat bottom, preferably black (for fluorometric assay)
- Dounce homogenizer (if using tissue)
- Deproteinizing Sample Preparation Kit – TCA (ab204708): for deproteinization step in cell or tissue samples
- 10 kD Spin Columns (ab93349): for deproteinization step in fluid samples

Reagent Preparation: Briefly centrifuge small vials at low speed prior to opening.

1. **Assay Buffer II/Glucose Assay Buffer:**
   Ready to use as supplied. Equilibrate to room temperature prior to use. Store at -20°C.

2. **OxiRed Probe/Glucose Probe:**
   Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. NOTE: DMSO tends to be solid when stored at - 20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Aliquot OxiRed Probe/probe so that you have enough volume to perform the desired number of assays. Store at - 20°C protected from light. Once the OxiRed Probe/probe is thawed, use within two months.
3. **Development Enzyme Mix II/Glucose Enzyme Mix:**
   Reconstitute in 220 µL Assay Buffer II/Glucose Assay Buffer. Keep on ice during the assay. Aliquot Development Enzyme Mix II/enzyme so that you have enough volume to perform the desired number of assays. Store aliquots at - 20°C. Use within two months.
4. **Glucose Standard (100 nmol/µL):**

Ready to use as supplied. Equilibrate to room temperature prior to use. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at - 20°C.

Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard the working standard dilutions after use as they do not store well.

For Colormetric Assay

- Prepare a 1 mL of 1 nmol/µL Glucose standard by diluting 10 µL of the Glucose Standard in 990 µL of Assay Buffer II/Glucose Assay Buffer.
- Using 1 nmol/µL Glucose standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes.

For fluorometric assay

- Prepare a 0.1 nmoL/µL Glucose standard by diluting 100 µL of 1 nmol/µL Glucose Standard with 900 µL of Assay Buffer II/Glucose Assay Buffer.
- Using 0.1 nmoL/µL Glucose standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes.

∆ Note: - Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

Standard #	Glucose Standard (µL)	Assay Buffer II/Assay Buffer (µL)	Final volume standard in well (µL)	End amount Glucose (nmol/well)	
				Colorimetric	Fluorometric
1	0	150	50	0	0
2	6	144	50	2	0.2
3	12	138	50	4	0.4
4	18	132	50	6	0.6
5	24	126	50	8	0.8
6	30	120	50	10	1.0

Sample Preparation

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

Cell (adherent or suspension) samples:

1. Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 106 cells).
2. Wash cells with cold PBS.
3. Resuspend cells in 100 µL of Assay Buffer II/Assay Buffer.
4. Homogenize cells quickly by pipetting up and down a few times.
5. Centrifuge 2 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.
8. Cell samples may contain enzymes that can interfere with the assay. Remove these enzymes from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708).

Tissue Samples:

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg tissue).
2. Wash tissue in cold PBS.
3. Resuspend tissue in 100 µL of Assay Buffer II/Assay Buffer.
4. Homogenize tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.
5. Centrifuge sample for 2 – 5 minutes at top speed at 4°C in a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.
8. Tissue samples may contain enzymes that can interfere with the assay. Remove these enzymes from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708).

Plasma, serum, urine and other biological fluids:

Add 2-50 µl test samples to a 96-well plate. Adjust the volume to 50 µl/well with Assay Buffer II/Glucose Assay Buffer. If using serum, limit sample volume to 0.5-2 µl/assay. Normal serum contains ~5 nmol/µl glucose. Urine can be assayed directly. Adjust the final volume to 50 µl with Glucose Assay buffer.

Biological fluid samples generally contain high amount of proteins which can interfere with the assay. Remove these enzymes from sample by using a 10kD Spin column (ab93349). Add sample to the spin column, centrifuge at 10,000 x g for 10 minutes at 4°C and collect the filtrate.

Alternative deproteinization protocol:

For this step you will need additional reagents:

- Perchloric acid (PCA) 4M, ice cold
- Potassium hydroxide (KOH), 2M

Δ **Note:** Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

1. Add PCA to a final concentration of 1M in the homogenate solution and vortex briefly to mix well. NOTE: high protein concentration samples might need more PCA.
2. Incubate on ice for 5 minutes.
3. Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.
4. Precipitate excess PCA by adding ice-cold 2M KOH that equals 34% of the supernatant to your sample (for instance, 34 µL of 2 M KOH to 100 µL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA.
5. After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust pH with 0.1 M KOH or PCA.
6. Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.
7. Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

Sample Recovery

- The deproteinized samples will be diluted from the original concentration.
- To calculate the dilution factor of your final sample, simply apply the following formula:

$$\%\ original\ concentration = \frac{initial\ sample\ volume\ )}{initial\ sample\ volume + volume\ PCA + volume\ KOH} x100$$

- Δ **Note:** We suggest using different volumes of sample to ensure readings are within the standard curve range.
  Endogenous compounds in the sample may interfere with the reaction. To ensure accurate determination of Glucose in the test samples, we recommend spiking samples with a known amount of Standard (4 nmol).

Assay Procedure – Colorimetric & Fluorometric Assay

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

†If you suspect your samples contain sucrose, set up Sample Background Controls

Plate Loading (clear plate):

For colorimetric use clear plates and for Fluorometric use black walled, clear bottom plates.

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2-50 µL samples (adjust volume to 50 µL/well with Assay Buffer II/Glucose Assay Buffer).
- Sample Background Control wells = 2-50 µL samples (adjust volume to 50 µL/well with Assay Buffer II/Glucose Assay Buffer).

The need to run background controls for every sample depends on sample type and background signal. i.e. †For uniform samples, it may be possible to run controls for only one representative sample. Run background control wells, each time you run the assay.

Glucose reaction mix:

1. Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number reactions +1).

Component	Reaction Mix (µL)		Background Reaction Mix (µL)	
	Colorimetric	Fluorometric	Colorimetric	Fluorometric
Assay Buffer II/Glucose Assay Buffer	46	47.6	48	49.6
OxiRed Probe/Glucose Probe	2	0.4	2	0.4
Development Enzyme Mix II/Glucose Enzyme Mix	2	2	0	0

2. Add 50 µL of Reaction Mix into each standard and sample wells.
3. Add 50 µL of Background Reaction Mix into the background control sample wells.
4. Mix and incubate at 37°C for 30 min protected from light.
5. Colorimetric: Measure output on a microplate reader at OD 570 nm.
6. Fluorometric: Measure output on a microplate reader at Ex/Em =535/587 nm.

Δ **Note: For fluorometric assays** using 0.4µL/well of the OxiRed Probe/Glucose probe decreases the background readings, therefore increasing detection sensitivity.

Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

- Average the duplicate reading for each standard and sample.
- If the sample background control is significant, then subtract the sample background control from sample reading.
- Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
- Plot the corrected absorbance values for each standard as a function of the final concentration of Glucose.
- Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
- Concentration of glucose in the test samples is calculated as:

$$\text{Glucose concentration} = \left(\frac{Sa}{Sv}\right) xD$$

Where:

Sa = amount of Glucose in the sample well calculated from the standard curve (nmol).
Sv = volume of sample added into the reaction well (µL).
D = sample dilution factor.

Glucose molecular weight: 180.2 g/mol.

- For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.
- For spiked samples, the concentration of Glucose in sample well is calculated as:

$$Glucose = \left(\frac{(ODs\ cor)}{(ODs + Sa\ cor) - (ODs\ cor)}\right) * Glucose\ spike\ (nmol)$$

Where:

ODs cor = OD sample corrected
ODs = OD sample
Sa cor = Glucose amount from standard

Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use provided protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer (increase number of strokes); observe for lysis under microscope
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Sample with erratic readings	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes and prepare a master mix whenever possible

	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so as to be in the linear range

Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure: Sucrose, Peroxide or Reducing agents. If sample contains reducing substances, we recommend using Glucose Detection Kit II (ab102517).

FAQs

Will the phenol red in the media affect the assay readout?

Very low amounts of media are used for each sample. This will generate a very low background at the best. Please use only media as a background control and subtract this reading from all sample readings to accommodate for the phenol red.

Why does the final color development starts as pink, then goes to brown then disappears?

This is a very common phenomenon observed with use of the OxiRed probe (contained in the Glucose Probe) and is caused due to excessive analyte concentration in the samples. Therefore, if your glucose samples are too concentrated, you may see this. Please dilute your samples with the Assay Buffer II/assay buffer before reanalyzing.

Will this kit detect glucose in purified polysaccharide samples?

This kit has not been tested in purified polysaccharide samples. The first step of the reaction is specific for glucose so it should be possible. However, background might be an issue so we recommend doing a background control check.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
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www.abcam.co.jp/contactus (Japan)