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ab176722 Glucose 6 Phosphate Dehydrogenase Activity Assay Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of glucose 6 phosphate dehydrogenase (G6PD) activity in various samples.

View kit datasheet: www.abcam.com/ab176722
(use www.abcam.cn/ab176722 for China, or www.abcam.co.jp/ab176722 for Japan)

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

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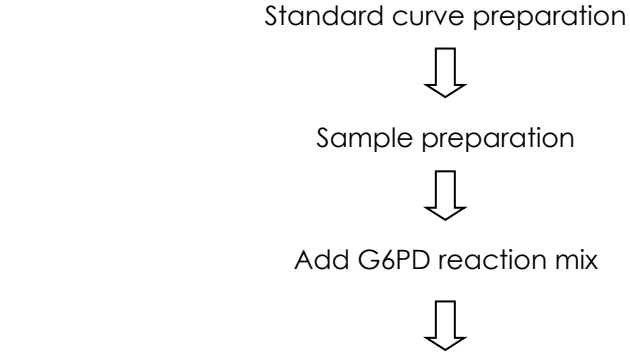
1. Overview

Glucose 6 Phosphate Dehydrogenase Activity Assay Kit (Fluorometric) (ab176722) provides a simple method for detecting glucose 6 phosphate dehydrogenase (G6PD) activity in a variety of biological samples such as serum, plasma and urine, as well as cell extracts. In the assay, G6PD present in the sample converts $NADP^+$ to NADPH, which can be specifically monitored by a fluorogenic NADPH sensor to yield a highly red fluorescence product. The signal can be easily detected at Ex/Em = 540/590 nm in a fluorescent microplate reader. The fluorescence intensity is proportional to the G6PD activity present in the sample.

The assay can detect glucose 6 phosphate dehydrogenase activity from as low as 0.3 mU/mL in a 100 µL reaction volume.

Glucose 6 Phosphate Dehydrogenase (G6PD, G6PDH, EC 1.1.1.49) catalyzes the conversion of glucose-6-phosphate to 6-phosphoglucono-δ-lactone, the first and rate-limiting step in the pentose phosphate pathway (PPP). It is a critical metabolic pathway that supplies reducing energy to cells (such as erythrocytes) by maintaining the level of co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The production of NADPH is of great importance for tissues actively engaged in biosynthesis of fatty acids and/or isoprenoids, such as the liver, mammary glands, adipose tissue, and the adrenal glands. The NADPH also maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage. Deficiencies in G6PD predispose individuals to non-immune hemolytic anemia.

2. Protocol Summary



**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. 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.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

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

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer	10 mL	-20°C	-20°C
G6PD Standard (10 U)	1 vial	-20°C	-20°C
Enzyme Probe (lyophilized)	2 bottles	-20°C	-20°C
NADP	1 vial	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 540/590 nm
- Double distilled water (ddH_2O)
- 1X PBS
- 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, preferably black

For cell lysate preparation:

- Cell scraper (for adherent cells)
- (Optional – if not using PBS) Mammalian Cell Lysis Buffer 5X (ab179835): for lysis of mammalian cells

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 G6PD Standard:

Reconstitute the G6PD Standard (10 U) in 100 µL of ddH_2O or PBS to generate a 100 u/mL G6PD standard stock solution. Keep on ice while in use. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C.

9.3 Enzyme Probe:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.4 NADP:

Dissolve the content of the vial in 100 µL ddH_2O to make a NADP Stock Solution (100X). Keep on ice while in use. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C.

10. Standard Preparation

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

10.1 Prepare 10 mL of 100 mU/mL G6PD standard solution by diluting 10 µL of G6PD Standard Stock Solution in 9990 µL 1X PBS.

10.2 Using 100 mU/mL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes to perform 1:3 and 1:3.3 serial dilutions:

Standard #	Sample to dilute	Volume standard in well (µL)	PBS Buffer (µL)	End activity G6PD in well (mU/mL)
1	100 mU/mL	200	400	33.33
2	Std #1	150	300	11.11
3	Std #2	150	300	3.70
4	Std #3	150	300	1.23
5	Std #4	150	300	0.41
6	Std #5	150	300	0.13
7	Std #6	150	300	0.04
8 (blank)	0	0	200	0

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Sample Preparation

General sample information:

- 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 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 and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Samples prepared by other protocols can be used as well for this assay. Do not use RIPA buffer as it will interfere with the assay. If you have your samples ready, please skip this section and proceed to Assay Procedure section.

11.1 Cell lysates:

Δ Note: For ease of use, mammalian adherent or suspension cells lysates can be easily prepared using Mammalian Cell Lysis Buffer 5X (ab179835). Follow product protocol and proceed to Section 12.

11.1.1 Harvest the number of cells necessary for each assay (initial recommendation: 2-5 x 10^5 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend or scrape cells in 100 µL of cold PBS.
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Centrifuge 5 minutes at 4°C at 13,000 x*g* in a cold microcentrifuge to remove any insoluble material.
11.1.6 Collect supernatant and transfer to a new tube.
11.1.7 Keep on ice.

11.2 Plasma, Serum and Urine (and other biological fluids):

Use heparin or EDTA as anticoagulant to collect plasma or serum. No sample preparation is required. Use directly or dilute in PBS.

11.3 Red Blood Cells (RBC):

11.3.1 Collect blood using heparin or EDTA as anticoagulant.
11.3.2 Centrifuge sample at 1,000 x*g* for 10 minutes at 4°C using a cold microcentrifuge. Discard supernatant.
11.3.3 Dilute RBC pellet 1:1 with PBS (pH 7.4) (ie, 1 mL RBC + 1 mL PBS. Place tube on ice.
11.3.4 Sonicate RBC with a few short bursts to break open the cells on ice.
11.3.5 Dilute RBC lysates 1:10-1:20 with PBS.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.
- The protocol describe in this section is for 1 x 96-well plate. To perform the assay in a 384-wp, scale down volumes by half.

12.1 Prepare assay reactions:

12.1.1 Add 5 mL of Assay Buffer into one bottle of Enzyme Probe (Step 9.3). Mix well by pipetting up and down.

12.1.2 Add 50 µL NADP Stock Solution (100X) to the mix to make a G6PD Assay Mixture. Mix well.

Δ Note: This assay mixture is enough for 1 x 96-well plate. Aliquot the unused assay mixture so that you have enough to perform the desired number of assays. Store at -20°C, kept from light.

12.2 Set up reaction wells:

- Blank control = 50 µL PBS.
- Standard wells = 50 µL standard dilutions.
- Sample wells = 1-50 µL samples (adjust volume to 50 µL/well with PBS).

12.3 Glucose 6 phosphate dehydrogenase assay reaction:

12.3.1 Add 50 µL of G6PD Assay Mixture(Step 12.1.2) into each well.

12.4 Measurement:

12.4.1 Monitor fluorescence increase at Ex/Em = 540/590 nm on a microplate reader in kinetic mode, every 5-10 minutes, for at least 30 minutes – 2 hours, at room temperature, protected from light.

Δ Note: Incubation time depends on the G6PD activity in the samples. We recommend measuring fluorescence in a kinetic mode and choosing two time points (T1 and T2) to calculate the G6PD activity of the samples.

13. Data Analysis

– 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.

13.1 Determination of reaction rate:

13.1.1 Subtract the fluorescence value of the blank (Standard #8) from all standard and sample readings. This is the corrected fluorescence.

13.1.2 For all wells (standard and samples), choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding RFU values at those points (RFU1 and RFU2)

13.1.3 Calculate reaction rate (ΔRFU/min) as follows:

$$\Delta RFU = (RFU2 – RFU1)/ (T2 – T1)$$

13.1.4 Average the duplicate reading for each standard and sample.

13.1.5 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step).

13.1.6 Plot the standard readings as function of the G6PD activity (mU/mL) and draw the line of the best fit (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of Glucose 6 phosphate dehydrogenase activity in the sample:

13.2.1 Interpolate G6PD activity (mU/mL) on the test samples from the standard curve data using the trend line equation.

13.2.2 Calculate G6PD activity in your sample as follows:

$$G6PD\ Activity = B * \left(\frac{Vwell}{Vsample}\right) * D$$

Where:

B = G6PD activity in sample well calculated from standard curve.

Vwell = total volume of well after reaction.

Vsample = original sample volume added into the reaction well.

D = sample dilution factor if sample is diluted to fit within the standard curve range.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

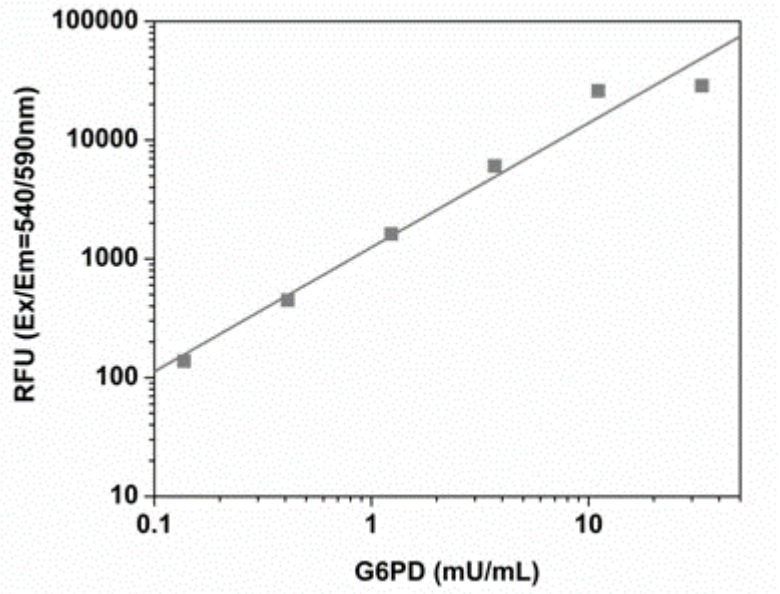


Figure 1. Typical Glucose 6 phosphate dehydrogenase (G6PD) dose response curve. Fluorescence was measured on a 96-well black solid plate using a Gemini fluoresence microplate reader (Molecular Devices). As low as 0.3 mU/mL Glucose 6 Phosphate Dehydrogenase (G6PD) activity can be detected after 60 minutes incubation.

The concentration of the Glucose-6-Phosphate Dehydrogenase in X-axis is based on the 50 uL standard, the final in-well concentration should be 1/2 for each point.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready
- Prepare G6PD standard dilution [300-0.3 mU/mL]
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL) and samples (50 µL)
- Prepare G6PD working solution: 5 mL Assay Buffer + Enzyme Probe + 50 µL of NADP Stock Solution (100X)
- Add 50 µL G6PD working solution to all wells.
- Monitor fluorescence increase at Ex/Em 540/590 nm on a microplate reader in kinetic mode for 30 min-2 hours at RT protected from light.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	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
Lower/higher readings in samples and standards	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

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) 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 described in the 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 it is within the linear range

17. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA Buffer, or any other sample preparation buffer that contains SDS or Tween-20.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)