Version 15-04/24

User's Manual

G6PD ELISA Kit

REF **DEIAPY17719A**

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For the quantitative measurement of G6PD in serum, plasma, tissue homogenates, cell culture supernatants, and other biological tissues.

General Description

This gene encodes glucose-6-phosphate dehydrogenase. This protein is a cytosolic enzyme encoded by a housekeeping X-linked gene whose main function is to produce NADPH, a key electron donor in the defense against oxidizing agents and in reductive biosynthetic reactions. G6PD is remarkable for its genetic diversity. Many variants of G6PD, mostly produced from missense mutations, have been described with wide ranging levels of enzyme activity and associated clinical symptoms. G6PD deficiency may cause neonatal jaundice, acute hemolysis, or severe chronic non-spherocytic hemolytic anemia. Two transcript variants encoding different isoforms have been found for this gene.

Principles of Testing

CD's G6PD ELISA Kit is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for G6PD has been pre-coated onto a 96-well plate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for G6PD is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample G6PD captured in the well.

Reagents And Materials Provided

1. G6PD Microplate, 96 Wells (12×8 Well strips), -20°C for 6 months
2. G6PD Lyophilized Standard, 2 vials, -20°C for 6 months
3. 100× Biotinylated G6PD Detector Antibody, 1×120 µL, -20°C for 6 months
4. 100× Avidin-HRP Conjugate, 1×120 µL, -20°C for 6 months
5. Sample Diluent, 1×20 mL, -20°C for 6 months
6. Detector Antibody Diluent, 1×12 mL, -20°C for 6 months
7. Conjugate Diluent, 1×12 mL, -20°C for 6 months
8. 25× Wash Buffer, 1×30 mL, Store at 4°C for 6 months
9. Stop Solution, 1×10 mL, Store at 4°C for 6 months
10. TMB Substrate, 1×10 mL, Store at 4°C for 6 months

Materials Required But Not Supplied

1. Microplate reader capable of reading absorbance at 450 nm.
2. Automated plate washer (**optional**).
3. Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
4. Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
5. New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
6. Absorbent paper or paper toweling.
7. Distilled or deionized ultrapure water.
8. 37°C Incubator (**optional**)

Storage

Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for 6 months or until expiration date. Avoid any freeze/thaw cycles.

Specimen Collection And Preparation

1. Sample Preparation and Storage

Note:

Samples must be tested to determine if the kit is valid.

Store samples to be assayed at 4°C for 24 hours prior being assayed.

For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

A. General Sample Preparation Guidelines:

a. Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

b. Plasma: Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

c. Tissue Homogenates: Rinse 100 mg of tissue with 1× PBS, then homogenize in 1 mL of 1× PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge the homogenate for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

d. Cell Lysates: Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in PBS (1×) and ultrasonicate the cells 4 times (or freeze cells at ≤ -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.) Centrifuge at 1,500 x g for 10 minutes at 2 - 8°C to remove cellular debris.

e. Cell culture supernatants and other biological fluids: Remove particulates by centrifugation and assay

immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

f. Recombinant Proteins: Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g. antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

2. Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

a. Prior to performing the full experiment, test a serially diluted representative sample.

Alternately, a small pool of several samples can also be used with this same method if sample volume is limited.

or

Refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit

b. Dilute samples using Sample Diluent.

c. Mix diluted samples gently and thoroughly.

d. Pipetting less than 2 µL is not recommended for optimal assay accuracy.

Reagent Preparation

Equilibrate all materials to room temperature prior to use and use immediately.

1. Standard

Prepare a fresh standard curve for each assay performed. Reconstituted standards cannot be stored for later use. For further directions, please refer to the Certificate of Analysis.

2. 1× Biotinylated G6PD Detector Antibody

a. Prepare the 1× Biotinylated G6PD Detector Antibody immediately prior to use by diluting the 100× Biotinylated G6PD Detector Antibody 1:100 with Detector Antibody Diluent.

b. For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of 100× Biotinylated G6PD Detector Antibody to 990 µL Detector Antibody Diluent.

c. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1× concentration for future use.

3. 1× Avidin-HRP Conjugate

a. Prepare the 1× Avidin-HRP Conjugate immediately prior to use by diluting the 100× AvidinHRP Conjugate 1:100 with Conjugate Diluent.

b. For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of 100× Avidin-HRP Conjugate to 990 µL Conjugate Diluent.

c. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1× concentration for future use.

4. 1× Wash Buffer

a. If crystals have formed in the 25× Wash Buffer concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

b. Add the entire 30 mL contents of the 25× Wash Buffer bottle to 720 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

c. Seal and mix gently by inversion. Avoid foaming or bubbles.

d. Store the 1× Wash Buffer at room temperature until ready to use in the procedure. Store the prepared 1× Wash Buffer at 4°C for no longer than 1 week. Do not freeze.

5. Microplate Preparation

a. Micro-plates are provided ready to use and do not require rinsing or blocking.

b. Unused well strips should be returned to the original packaging, sealed and stored at 4°C.

c. Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

Assay Procedure

Note:

a. Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

b. Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

Procedure:

1. Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.
2. Add 100 µL of serially titrated standards, diluted samples or blank into wells of the G6PD Microplate. At least two replicates of each standard, sample or blank is recommended.
3. Cover the plate with the well plate sealer and incubate at 37°C for 2 hours.
4. Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
5. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
6. Add 100 µL of prepared 1× Biotinylated G6PD Detector Antibody to each well.
7. Cover with the well-plate sealer and incubate at 37°C for 60 minutes.
8. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
9. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
10. Wash plate 3 times with 1× Wash Buffer as follows:

a. Add 300 µL of 1× Wash Buffer to each assay well.

b. Incubate for 1 minute.

c. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

d. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling.

Do not allow the wells to completely dry at any time.

e. Repeat steps 10.10.1 through step 10.d two more times.

11. Add 100 µL of prepared 1× Avidin-HRP Conjugate into each well, cover with plate sealer and incubate at 37°C for 60 minutes.
12. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
13. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
14. Wash plate 5 times with 1× Wash Buffer as in Step 10.
15. Add 90 µL of TMB Substrate to each well, cover with plate sealer and incubate at 37°C in the dark for 15-30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(**NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.**)

16. Add 50 µL of Stop Solution to each well. Well color should change to yellow immediately. Add the Stop Solution in the same well order as done for the TMB Substrate.
17. Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 16. If wavelength correction is available, set to 540 nm or 570 nm.

Calculation

For analysis of the assay results, calculate the Relative OD450 for each test or standard well as follows:

(Relative OD450) = (Well OD450) – (Mean Blank Well OD450)

The standard curve is generated by plotting the mean replicate Relative OD450 of each standard serial dilution point vs. the respective standard concentration. The concentration contained in the samples can be interpolated by using linear regression of each mean sample Relative OD450 against the standard curve. This is best achieved using curve fitting software. A standard curve should be generated each time the test is performed.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

Specificity

Human G6PD.

No detectable cross-reactivity with other relevant proteins.

Precautions

1. Read instructions fully prior to beginning use of the assay kit.
2. Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

3. Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
4. Kit cannot be used beyond the expiration date on the label.

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