| PATH | Standard Operating Procedure | |
|---|---|---|
| **Document No: SPR-xx** | **Version Number:** [Version No.] | **Date signed:** [Effective Date] |
| **Title:** | **Trinity Biotech Glucose-6 Phosphate Dehydrogenase Quantitative Test** | |

## 1. Purpose

The Trinity Biotech Glucose-6 Phosphate Dehydrogenase assay kit is used for the quantitative, spectrophotometric determination of G-6-PDH activity in human blood samples.

## 2. Scope

This SOP applies to quantitative screening of human red blood cells specimen for determining G6PD enzyme activity for successful evaluation and development of other G6PD Point of care diagnostic tests.

## 3. Responsibilities

3.1. The Project lead has the authority to establish this procedure.

3.2. The Scientific lead is responsible for the control of SOP documentation.

3.3. Laboratory staff is responsible for the implementation of this procedure and for ensuring that all appropriate personnel are trained.

## 4. Health and Safety Information

4.1. Consider all patient specimens as capable of transmitting infectious agents. Use appropriate precautions such as gloves, safety glasses, and laboratory coats when collecting, handling, and disposing of patient specimens.

4.2. Dispose of all specimens and used materials in accordance with local applicable guidelines and/or regulations.

4.3. Refer to MSDS for the Trinity G6PDH assay more information.

## 5. Required Equipment/Materials

5.1. Shimadzu UV-1800 spectrophotometer with temperature control unit

5.2. UV-transparent disposable plastic cuvettes, Brand part # 7591-50

5.3. Ultra pure water

5.4. Water bath

5.5. Pipettes and tips capable of accurately dispensing 10 µl and 1 ml.

5.6. Disposable test tubes capable of holding 3 ml, with caps

5.7. Vortex mixer
5.8. Delicate-task tissues (eg. Kimwipes)
5.9. Trinity Biotech Quantitative assay kit, part # 345-B
5.10. Trinity Biotech Quantitative assay kit product insert
5.11. Digital timer

## 6. Optional Materials

6.1. G6PDH deficient control, Trinity Biotech part # G5888
6.2. G6PDH intermediate control, Trinity Biotech part # G5029
6.3. G6PDH normal control, Trinity Biotech part # G6888

## 7. Preliminary Operations

7.1. Carefully read the product insert completely before using the test. Use the test only in accordance with the instructions contained in the product insert. Failure to do so may result in inaccurate test results.
7.2. Do not use the kit contents after the expiration date listed on the kit.
7.3. Allow water bath to pre-warm for at least 30 minutes.
7.4. Prepare G-6-PDH assay solution by adding a volume of ultra pure water indicated on the label to the reagent vial. Swirl gently and invert several times. Let stand for 3 minutes and mix again.

## 8. Optional Preliminary Operations

8.1. The assay can be calibrated when needed by testing deficient, intermediate, and normal controls, provided by Trinity Biotech. To test the controls, first rehydrate with 0.5 ml pure water. Mix well, and visually verify blood has been rehydrated before using. Follow the procedure with controls exactly as with whole blood specimens. Compare calculated G6PD activity values with the expected value ranges provided by Trinity Biotech.

## 9. Procedure

9.1. Add 1.0 ml reconstituted G-6-PDH assay solution (from kit) to test tube.
9.2. Add 10 µl of blood to test tube containing assay solution. Mix thoroughly by vortex. Let stand at room temperature for 5 minutes.

9.3. Push F4 button on spectrophotometer to enable computer control. Open UVProbe program on attached computer. Click "connect" button near the bottom of the screen. Click the "file" tab and select "open". Change file type to "method". Open "5 minute 6 cell" file in "Methods" folder. Absorbance should be read at 340 nm.

9.4. Place a plastic cuvette containing pure water into cell position 1. Click "auto-zero" tab near the bottom of the screen.

9.5. Add 2.0 ml G-6-PDH substrate solution (from kit) to test tube containing assay solution and blood. Mix by inverting tube several times.

9.6. Aliquot solution from test tube into one to three plastic cuvettes, 1 ml each. Place cuvettes in constant temperature water bath set to 30.5° C. Incubate for exactly 5 minutes.

9.7. Remove cuvettes from water bath and dry with tissues. Place immediately into temperature-controlled spectrophotometer set to 31.1° C.

9.8. Click "start" button near the bottom of the screen. Rapidly click "next" once for each cell being tested.

9.9. When the spectrophotometer completes the programmed cycle, click "point pick" button near the top of the screen (26$^{th}$ button from the right). Enter desired time point for initial reading, in seconds. Record $A_{initial}$. Enter a second time point 308 seconds after the first time point. Record $A_{final}$. (For example, the first cell will likely be read at $T_{second}$ = 1, 308).

## 10. Result calculations

10.1. Calculate results using formula given in Trinity kit insert:

Where

- 100 = factor to convert activity to 100 ml
- 3.01 = total reaction volume (ml)
- 0.01 = sample volume (ml)
- 6.22 = millimolar absorptivity of NADPH at 340 nm
- Hb (g/dl) = hemoglobin concentration determined for each specimen

$\Delta A = A_{final} - A_{initial}$

$$\text{G-6-PDH (U/g Hb)} = \Delta A\ per\ \min \times \frac{100 \times 3.01}{0.01 \times 6.22 \times Hb\left(^{g}/_{dl}\right)}$$

$= \Delta A\ per\ \min \times \frac{4839}{Hb(^{g}/_{dl})}$

**Example calculation**:

Assume $A_{initial}$ is 0.9515, and $A_{final}$ is 1.012, and Hb concentration is 11.1 g/dl:

ΔA = 1.012 – 0.9515
ΔA = 0.0605
ΔA per min = 0.0605 / 5 = 0.0121
G-6-PDH (U/g Hb) = 0.0121 x ((100 x 3.01) / (0.01 x 6.22 x 11.1))
**G-6-PDH (U/g Hb) = 5.275**

## 11. Storage

All reagents should be refrigerated (2-8° C) when not in use. Reconstituted assay solution is stable for 5 days refrigerated.

## 12. Related SOPs

**12.1.** Sample receiving, handling and data management
**12.2.** Sample shipment from specimen repository

**SIGNATURES:**

_____
_____
Date

_____
_____
Date

_____
_____
Date