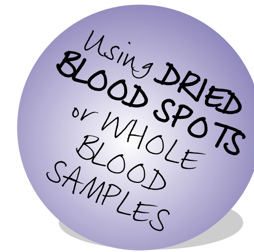
G-6-PD Screening assay

Enzymatic colorimetric assay for the quantitative determination of Glucose-6-Phosphate Dehydrogenase activity



Indications

This test is intended for use as a neo-natal screening method for red cell Glucose-6-Phosphate Dehydrogenase deficiency in newborns.

Features

- One step test using a unique Hb normalization procedure
- Easily adapted to automated analysers
- Convenient transport and good stability of the dried blood samples

Kit contents

- **100 tests**
  - Reagent vials : 4 x 25 tests
  - Elution buffer : 1 x 8 ml
- **2000 tests**
  - Reagent vials : 20 x 100 tests
  - Elution buffer : 4 x 40 ml

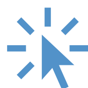
Also available

- G6PD UV 500 tests for the qualitative determination of G-6-PD activity under special UV lamps (Code E-II-500)
- G6PD 550nm 2000 tests for the quantitative determination of G-6-PD activity with reading at 550nm (Code E-IL-2000)

ORDERING INFORMATION

Code: ***E-HO-100***
Package Size : 100 tests/kit

Code: ***E-HO-2000***
Package Size : 2000 tests/kit



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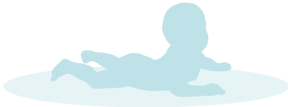
NEONATAL SCREENING



G-6-PD
Screening assay
Simple assay procedure

1. Take a clean 96-well (preferably U bottom) microplate (elution microplate).
2. Add 5 µl of whole blood or dried blood spot (4.7 mm or 2 X 3.2 mm) per well. Whole blood samples and dried blood spots can be measured simultaneously. Remember to add controls (not included in this kit)*.
3. Add 75 µl of Elution Buffer in each well, mix well the contents of each well and place the plate in a plate shaker.
4. Incubate 30 minutes at room temperature in a plate shaker.
5. Reconstitute one Reagent vial with 8 mL distilled water if using the 2000 tests/kit format or 2 mL if using the 100 tests/kit format (stable for at least one week refrigerated).
6. Transfer 15 µL of the eluant from each well to the corresponding well of a new flat bottom microtiter plate.
7. Add 75 µl of reagent to each well and mix well.
8. Place the plate on an orbital shaker for 1 minute.
9. Place the reagent microplate in the incubator or reader and wait for 10 minutes (for assay temperature 22-25 °C) or 7 minutes (assay temperature 25-37 °C).
10. Read the plate at 340 nm for 15-20 minutes with 60 seconds intervals. Alternatively, the NADPH produced can be measured in endpoint mode using two measurements one at time=0 and a second one at time=10-15 minutes later.
11. After the readings are completed, change the program of the reader to an endpoint mode, select wavelength = 405 nm and read the plate once again.
12. Use the following formula to express your results directly in U/g Hb.

For « endpoint mode » : two single readings :

$$\frac{(\delta\ \text{OD sample}_{340nm})/(\delta\ \text{OD control}_{340nm})}{\text{OD sample}_{405nm}/\text{ODcontrol}_{405nm}} \times \text{Control Value} = \text{Sample Value}$$

(Activity In U/g Hb)

For « kinetic mode » : 10-15 measurements, on minute intervals :

$$\frac{(\delta\ \text{OD sample}_{340nm}/\text{min})/(\delta\ \text{ODcontrol}_{340nm}/\text{min})}{\text{Od sample}_{405nm}/\text{OD control}_{405nm}} \times \text{Control Value} = \text{Sample Value}$$

(Activity in U/g Hb)

Description of the formula

δ OD sample **is the change in optical density (total change or change per minute depending on the reading mode) for the sample at 340 nm,** δ **OD control is the change in optical density** (total change or change per minute depending on the reading mode) of the control at 340 nm, OD sample is the optical density for the sample measured once at 405nm and OD control is the optical density of the control measured once at 405nm. You must substitute the "Control Value" with the value of the control expressed in U/g Hb.

*We recommend to use the **Sigma® Chemical Co G-6-PDH** controls.

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