G6PD Assay Kit - WST

100 assays, 500 assays

Technical Manual (in Japanese) is available at http://www.dojindo.co.jp/manual/g256.html

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, the most common enzymopathy, causes acute hemolytic anemia. Therefore the measurement of G6PD activity is important, particularly in malaria-endemic areas where primaquine is widely used as an anti-malaria drug in the treatment of malaria. G6PD Assay Kit-WST utilizes a water-soluble tetrazolium salt, WST-8, that also produces water-soluble formazan with an intense orange color at 460 nm. WST-8 does not react with hemoglobin, allowing a very simple and rapid assay for screening of G6PD activity.

Kit Components

	for 100 assays	for 500 assays
Substrate Mixture	2 ml x 1 vial	2 ml x 5 vials
Dye Mixture	2 ml x 1 vial	2 ml x 5 vials

Required Equipment and Materials

20 μl and 1 ml micropipettes and tips
1.5 ml microcentrifugation tubes
water (commercially available, fresh drinking water)
1 mol/l HCl

Assay

1. Add sequentially 760 μl of water, 20 μl of Substrate Mixture and 20 μl of Dye Mixture into 1.5 ml tubes.
2. Add 5 μl of sample bloods into 1.5 ml tubes.
3. Mix them vigorously for 5 sec and incubate at 25~37°C for 20~30 min.
4. Stop the reaction by adding 10 μl of 1 mol/l HCl and compare the developed color with those of positive and negative controls.

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Revised February 15, 2017

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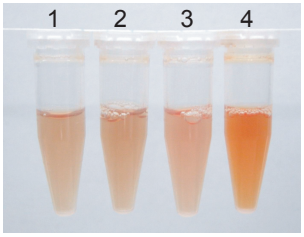
Orange color development (HCl is not added).



1: nagative control
normal blood without substrate
(G6PD activity 0%)
2: heterozygous male
(0% activity)
3: heterozygous female
(50% activity)
4: normal blood,(8 IU/g Hb)
(100% activity)
Reaction: 25°C for 30 min

Notes

1) Store the Kit at -20°C. This kit is stable for 6 months at -20 °C, for one month at 4°C, and for ten days at 37°C .
2) After thawing Substrate Mixture and Dye Mixture, it is recommended to mix them with 76 ml of water (total 80 ml) and dispense 800 µl of the mixture to 1.5 ml tubes as described in the assay procedure. The mixture is stable for 2 weeks at -20°C and 3 days at 4°C with protection from light.
3) Hydrochloric acid as a reaction stop solution also helps to distinguish the color-difference.
4) As a standard negative control, 5 µl of G6PD-normal blood in the reaction mixture without the substrate can be used.
5) The activity can be quantified by measuring absorbance at 450-460 nm with a microplate reader. For accurate diagnosis, measurement of hemoglobin content is recommended.
6) This kit is for research use only.

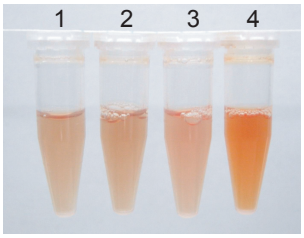
Reference

I.S. Tantular and F. Kawamoto, *Trop. Med. Int. Health*, **2003**, *8* (6), 569.

DOJINDO Laboratories
Telephone: +81-96-286-1515
Facsimile: +81-96-286-1525
Web site: www.dojindo.co.jp/

G6PD Assay Kit - WST
General Protocol - page 2

Orange color development (HCl is not added).



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