Determination of total glutathione (GSH) and oxidized glutathione (GSSG) levels of RBCs infected with Plasmodium falciparum (Pf RBCs)

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Abstract

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Protocol

Step 1.

step 1

Preparations of PfRBCs for total GSH and GSSG estimation:

1. Harvest PfRBCs*/RBCs (5.4 x 10^8 cells) in cultures and washed with phosphate-buffered saline.

*at 5% parasitemia

2. Disrupt the packed PfRBCs/RBCs by three freeze-thaw cycles.
3. Add 100 µl 5% (w/v) sulfosalicylic acid solution (SSA) to the disrupted PfRBCs/RBCs, and vortex.
4. Collect the supernatants by centrifugation for 5 min at 8,000g at 4°C, and transfer to 500 µl chilled, ultrapure water* (final volume 600 µl).

*Wako Pure Chemical Industries, Osaka, Japan, catalogue no. 210-01303

Step 2.

step 2

Quantification of total glutathione (GSH + GSSG) and GSSG by using the total Glutathione Quantification kit or the GSSG/GSH Quantification kit*.

*total Glutathione Quantification kit (catalogue no. T419) and GSSG/GSH Quantification kit (catalogue no. G257)

Dojindo Molecular Technologies, Inc., Rockville, MD, USA

Step 3.

step 3

Quantification of total glutathione (GSH + GSSG) and GSSG using the GSSG/GSH Quantification kit (G257)

1. For total GSH estimation, prepare GSH standard solutions in 0.5% SSA (50, 25.0, 12.5, 6.25, 3.13, 1.57, 0 µM).
2. For GSSG estimation, prepare GSSG standard solutions in 0.5% SSA (25.0, 12.5, 6.25, 3.13, 1.57, 0.78, 0 µM).
3. Add 4 µl masking solution to 200 µl GSSG standard solutions, and 200 µl samples prepared by the procedure above (step 1) in a micro tube, and mix well.
4. Place 40 µl of GSSG standards, GSH standards, and samples in a 96-well microtiter plate.
5. Add 60 µl Buffer solution to each well.
6. Incubate the plate at 37 °C for 1 h.
7. Add 60 µl of Substrate working solution to each well.
8. Add 60 µl of Enzyme/coenzyme working solution to each well, and mix well.
9. Incubate the plate at 37 °C for 10 min.
10. Read the absorbance at 405 nm (OD_{405}) on a microplate reader, and extrapolate concentrations of total GSH and GSSG from each standard curve.