APPENDIX A

Media and reagents for parasite culture

1. RPMI solution (serum-free RPMI)

The RPMI medium was prepared as a stock solution by mixing the following ingredients 1 L distilled water (DW):

RPMI 1640 (Biochrom, Germany)	10.43 g
Sodium hydrogen carbonate	2.00 g

This mixture was filtrated through 0.22 µm acrylic membrane of bottle top filter set (Corning, U.S.A.) for sterilizing and then stored at 4°C until used.

2. 1M HEPES buffer

This buffer was prepared by dissolving 23.831 g of N-2-Hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES) (Sigma, U.S.A.) in 100 ml of DW. The solution was adjusted to pH 7.4 with 1 N HCl and sterilized by filtering through a sterile 0.2 µm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

3. Complete medium

To prepared complete medium, 5 ml of HEPES, 10% of AB or B group of serum and 300 µl 10 mg/ml Gentamicin were mix with 200 ml of serum free RPMI. The complete medium was stored at 4°C until used.

4. Phosphate buffer saline (PBS buffer pH 7.2)

This buffer was prepared by dissolving 1 tablet of PBS (Zymed, U.S.A.) in 100 ml of DW. The solution was sterilized by filtration through a sterile 0.2 µm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

5. 0.9% Sodium chloride (NaCl)

The solution was prepared by dissolving 4.5 g NaCl in 500 ml DW, and filtered through a sterile 0.2 µm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

6. Freezing solution

The freezing solution consisted of 7.56 g sorbital, 180 ml of 0.9% NaCl and 70 ml of 99% glycerol. The solution was steriled by filtering through a 0.45 µm Millipore membrane and kept at 4°C until used.

7. 3.5% Sodium Chloride (NaCl)

The solution was prepared by dissolving 3.5 g NaCl in 100 ml DW. The solution was steriled by filtering through a sterile 0.2 µm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

8. 5% Sorbital

The solution was prepared by dissolving 5 g of sorbital (Sigma, U.S.A.) in 100 ml DW and sterilized by filtering through a sterile 0.2 µm acrylic membrane (Ultra pure, AquaPor LM, U.S.A.) and stored at 4°C until used.

9. Non-infected erythrocytes

Preparing pack red cell group O from blood bank by transferred to 15 ml centrifuge tube and added with serum-free RPMI or PBS and centrifuge at 2000*x g*, 10 minutes, and then removed supernatant. This pack red cell was washed until clear of buffy coat or white blood cell and kept 4°C until used.

10. [^{3}H] Hypoxantine

This solution was prepared by made up 500 µl into 10,000 µl of complete medium that was twenty-fold dilution.

11. Preparation of drug solution for *in vitro* sensitivity tests

Each drug test was prepared as a stock drug solution of 10^{-2} nM

11.1 Chloroquine (CQ)

To prepared a stock CQ concentration of 10^{-2} nM, 51.59 mg of CQ diphosphate salt (Sigma, U.S.A.; MW = 515.9) was dissolved in 10 ml of 50% ethanol (HPLC grade).

11.2 Artesunate (ARS)

To prepared the stock ARS concentration of 10^{-2} nM, 38.44 mg of ARS (Dafra Pharma NV; MW = 384.4) was dissolved in 10 ml of 50% ethanol (HPLC grade).