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).

APPENDIX B

Reagents for study the effect of temperature and drug stress on *Plasmodium falciparum* heat shock protein 70 (*pf*HSP70s).

1. Electrophoresis

SDS-PAGE (Laemmli) Buffer system

Stock-Solution and Buffers

1.1 Acrylamide / Bis (30% T, 2.67% C)

Acrylamide	29.2 g
N'N'-bis-methylene-acrylamide	0.8 g

Make to 100 ml DW. Filter and store at 4°C in dark (30 Days maximum).

1.2 10% SDS (100 ml)

SDS	10 g

Dissolve 10 g SDS in 90 ml water gentle stirring and bring to 100 ml with DW.

1.3 1.5 M Tris-HCl, Ph 8.8 (100 ml)

Tris base	18.15 g
DW	50 ml

Adjust to pH 8.8 with 6 N HCl. Bring total volume to 100 ml with DW and store at 4°C.

1.4 0.5 M Tris-HCl pH 6.8 (100 ml)

Tris base	6 g
DW	50 ml

Adjust to Ph 6.8 with 6 N HCl. Bring total volume to 100 ml with DW and store at 4°C.

1.5 10x Electrode (Running) Buffer, pH 8.3 (1 L)

Tris base	30.3 g
Glycine	144.0 g
SDS	10.0 g

Dissolve and bring total volume up to 1,000 ml with DW. Do not adjust pH with acid or base, store at 4˚C. If precipitation occurs, warm to RT before use.

1.6 10% APS (Fresh daily)

Ammonium persulfate	100 mg

Dissolve in 1 ml of DW.

1.7 Sample Buffer (SDS Reducing Buffer)

DW	3.55 ml
0.5 M Tris-HCl, pH 6.8	1.25 ml
Glycerol	2.5 ml
10% SDS	2.0 ml
0.5% Bromophenol blue	0.2 ml
Total volume	9.5 ml

Store at Room temperature.

Add 50 ml Mercaptoethanol to 950 ml sample buffer prior to use.

Dilute the sample at least 1:2 with sample buffer and at 95˚C for 4 minutes.

2 Western Blot Analysis

Stock-Solution and Buffer

2.1 Transfer Buffer

25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3

Tris	3.03 g
Glycine	14.4 g
Methanol	200 ml

Add ddH_2O to 1 ml

All formulas provide below are for a total volume of 1 litter of buffer. Approximately 500 ml of buffer are required for the Mini Trans-Blot cell. Do not add acid or base to adjust pH of the following buffers. Methanol should be analytical reagent grade, as metallic contaminants in low grade methanol will plate on the electrodes.