



APPENDIX B

สำนักหอสมุด

**Reagents for study the effect of temperature and drug stress on
Plasmodium falciparum heat shock protein 70 (pfHSP70s).**

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
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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 ddH₂O to 1 ml

All formulas provide below are for a total volume of 1 liter 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.

