

**STANDARD OPERATING PROCEDURES**

**TITLE:** HRP2 DOUBLE-SITE SANDWICH ELISA FOR USE IN THE HRP2 DRUG SENSITIVITY ASSAY

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**Purpose and Scope:** This SOP describes a simple **double-site sandwich ELISA** based on 2 commercially available monoclonal antibodies and optimized for *P. falciparum* in vitro drug sensitivity testing (Noedl et al, AAC 2002, Noedl et al AJTMH 2004). Please see separate SOPs for sample collection/handling, drug dosing, and culture procedures (HRP2 lab test, HRP2 field test).

**Definitions:** NA

**Safety Concerns:** All safety precautions related to the handling of potentially infectious human and parasite material apply. Concentrated sulfuric acid is a strong acid with corrosive properties. The hydration reaction of sulfuric acid with water is highly exothermic.

**Specific Materials and Equipment:**

Product, Supplier, Product No.	Unit	Unit Cost List Price \$US (2004)	Approx. Cost per Plate
PBS, PH 7.4, Sigma-Aldrich, P3813	10 foil pouches for 1L each	31.30	0.9 for 300 mL
Tween 20, Sigma-Aldrich, P1379	500 mL	27.60	0.06 for 2 mL
BSA, Sigma-Aldrich, A9647	100g	170.8	1 for 0.6 g
TMB, Zymed Laboratories, Inc	500mL	Approx. 175 (Thailand)	3.5 for 10 mL
ELISA high binding 96 well plate Corning Inc., Costar 3590, 96 Well EIA/RIA	100 plates	Approx. 100 (Thailand)	1 per plate
Sulphuric Acid			
MPFM-55A, Immunology Consultants Laboratories, Inc	1 mg	225	2.25 for 10 ug
MPFG-55P Conjugate, Immunology Consultants Laboratories, Inc	0.5 mg	200	0.3 for 0.5 ug
		<b>TOTAL</b>	<b>Approx. \$US 9 List</b>

**Specific Procedures:****1.) Coating ELISA plates:**

- Dilute primary IgM antibody (MPFM-55A, Immunology Consultants Laboratories, Inc, Newberg, OR, USA) to **1.0 ug/mL in PBS** (if original solution as shipped from manufacturer is 11.5 mg/mL → dilute 9 µL of original solution in 103.5 mL PBS – 100 mL is sufficient for coating 10 plates).
- Transfer 100 µl of diluted antibody per well to the ELISA plate using a multichannel pipette.
- Seal plate and incubate at 4°C overnight. Discard contents of the ELISA plate and bang dry.
- Blocking solution: Prepare 2% bovine serum albumin (BSA) solution in PBS (For each plate dissolve 0.4 g of BSA (Sigma, CAS No. 9048-46-8) in 19.6 mL PBS).
- Add 200 µl of blocking solution per well and incubate at room temperature for 2 hours, discard, and bang dry.
- Prepare PBS/Tween (0.05%) washing solution: Add 0.5 ml Tween 20 to 999.5 mL PBS.
- Wash plate 3 times in PBS/Tween (200 µl/well), bang dry, seal airtight in plastic bag and freeze at -20°C.

**2.) ELISA Step 1: Add sample**

- Transfer 100 uL of sample from the culture plate to the ELISA plate (starting parasite density before culture should be equivalent to around 0.05 %, 1.5% hct – samples may be diluted before or after culture - **see separate SOPs for handling and diluting samples**).
- Incubate for 1 hour at room temperature in humid chamber
- Wash 3 times in PBS/Tween (200 µl/well) and bang dry

**3.) ELISA Step 2: Dilute antibody conjugate and add to ELISA plate**

- Diluent for 2<sup>nd</sup> antibody: Prepare solution of 2% BSA + 1% Tween 20 in 97 mL PBS (2 g BSA + 1 mL Tween 20 + 97 mL PBS – prepare 10 mL per plate) and preferably adjust to pH 7.4
- Dilute second antibody (MPFG-55P, Immunology Consultants Laboratories, Inc, Newberg, OR, USA) to **0.2 µg/mL (range 0.05 to 0.2 depending on the activity of the conjugate) in diluent. Caveat: The activity of the conjugate may vary from batch to batch. When using a new batch try different concentrations of the conjugate (titrate) to find optimal dilution!**
  - Prepare a **200x stock solution (40 µg/mL)** of the second antibody (do not store this stock solution for extended periods of time)
    - **Stock solution (200x):** if original solution as shipped from manufacturer is 1 mg/mL → dilute 40 µL of the original antibody solution in 1 mL diluent – this quantity will be sufficient for around 20

plates).

- **Final working solution:** For each plate add 50 µL of 200x antibody stock solution to 10 mL of diluent.
- Transfer 100 µl of diluted 2<sup>nd</sup> antibody per well to the ELISA plate using a multichannel pipette
- Incubate for 1 hour at room temperature in humid chamber
- Wash 3 times in PBS/Tween (200 µl/well) and bang dry

#### 4.) ELISA Step 3: Add substrate and read plate

- Add 100 µl per well of **TMB chromogen** (TMB Single Solution Chromogen, Zymed Lab., Inc., San Francisco, CA, USA) incubate for 5 to 10 minutes at room temperature in the dark.
- **Stop** reaction with 50 µl of 1 M sulphuric acid (add 10 mL of sulphuric acid to 90 mL of water to prepare the stop solution). **Caveat:** always add sulphuric acid to water, never the other way around!
- Read absorbance using an ELISA plate reader **at 450 nm**

#### References:

Noedl H, Bronnert J, Yingyuen K, Attlmayr B, Kollaritsch H, Fukuda M. Simple histidine-rich protein 2 double-site sandwich enzyme-linked immunosorbent assay for use in malaria drug sensitivity testing. *Antimicrob Agents Chemother.* 2005 Aug;49(8):3575-7.