



# Product Information Sheet for MRA-593

## PARASITE

**MR4 Number:** MRA-593

**Organism:** *Plasmodium yoelii*

**Strain:** 17XNL(1.1)

**Unit size:** 0.5 ml

**Original Preparation Contributed By:** Landau and Killick-Kendrick.

**Depositor:** Daniel Carucci

**Reference:** Weiss, W.R., M. Hollingdale, L.H. Miller, and J. A. Berzofsky. 1989. Genetic Control of Immunity to *Plasmodium yoelii* Sporozoites. *J. Immunol.* 143:4263-4266.

**Source of isolation:** Mouse (*Mus musculus*)

**Storage:** Ships on dry ice. Cryovials should be immediately transferred to a -70°C or below freezer for temporary storage (~2 weeks) or liquid nitrogen for long term storage.

### Cultivation protocol (MR4):

1. For recovery of cryopreserved parasites, thaw frozen vial at 37°C for approximately 2 minutes until contents of tube are fully thawed. Hold sample on ice while preparing for mouse infection. Infect mouse (BALB/c or appropriate strain) via interperitoneal (IP) injection of 0.1-0.15 ml of the thawed blood sample. Do not exceed 150 ul of the MR4 supplied cryopreserved stock. Cryopreserved samples should provide sufficient volume to infect 3 - 5 mice. (Optional) - make a blood smear slide using a small droplet (~10-20 ul) of the sample stock and stain by the Giemsa method to check and record the parasitemia of the sample.
2. From day 3, monitor parasitemia daily by taking small blood samples by tail vein bleed and make a blood smear for staining and microscopy. Evaluate the parasitemia each day until the parasitemia levels reach appropriate level; 10-30% parasitemia is common for many rodent malaria strains in mice while some *P. berghei* strains may not exceed 5-10%. Strains known to be virulent or lethal should be harvested or passaged at or before parasitemia exceeds 20%. Any animals showing significant signs of distress should be evaluated immediately for processing or euthanasia.
3. Anesthetize mice using facility approved protocols (anesthetic or low dose of inhaled CO<sub>2</sub>). Collect blood samples by Orbital Sinus Bleeding, cardiac puncture (terminal bleed) or facility approved method. Blood sample yield will vary depending on technique used. Exsanguinate the mice at the completion of the study following approved guidelines for your facility.
4. To maintain the parasite strain *in vivo*, passage infected blood from donor mouse to recipient mouse via IP route. One droplet, ~5 ul, of infected blood at a parasitemia between 5-15%, should be diluted in 10 ml of Phosphate Buffered Saline (PBS) and 100 ul of the suspension used to infect recipient mice by serial passage; at 10% parasitemia 5 ul is equivalent to ~3 x 10<sup>5</sup> infected RBC. By day 4-7 post injection the parasitemia should increase from 0.1 to 5-20% during serial passage, depending on inoculum, parasite and mouse strains used.

### Cryopreservation:

1. For cryopreservation, centrifuge the parasite infected blood at 2000 rpm (900 - 1000 x g) for 5 minutes.
2. Remove the supernatant and add 100% glycerolyte solution (GLYCEROLYTE 57 solution (Baxter); 6.2M buffered glycerine, lactate, and potassium solution; ~57% glycerol) in equal volume to that of packed cells. Add solution SLOWLY, drop by drop, at room temperature with gentle agitation and incubate at room temperature for 5 minutes to allow glycerol to penetrate cells.
3. Add 1 more volume of 100% glycerolyte drop wise. Final concentration of glycerol is ca. 38%.
4. Aliquot 0.5 ml in labeled ampoules and slow freeze (-1°C min<sup>-1</sup> at -80°C) in a freezing chamber (e.g. Mr. Frosty<sup>®</sup>, Nalge).
5. Following overnight storage in the -70°C freezer, transfer vials to the vapor or liquid phase in a liquid nitrogen tank for long term storage.

n.b. Glycerolyte 57 solution is composed of:  
Glycerol 57 g; Potassium Chloride 30 mg; Sodium Lactate 1.6 g;  
Sodium Phosphate Dibasic 124.2 mg; Sodium Phosphate Monobasic 51.7 mg per 100 ml dH<sub>2</sub>O; pH 6.8 adjusted with phosphoric acid.

**Quality Control:** BALB/c mouse was infected with 0.1 ml of parasites from a thawed vial of cryopreserved stock by IP injection and the parasitemia was monitored for 6 days. Typical parasitemia on day 4 for most stocks is 5%.

### References:

#### Important notes:

This reagent was authenticated by the contributor and amplified at MR4, ATCC. Please contact [malaria@atcc.org](mailto:malaria@atcc.org) for comment.

ALL BLOOD CULTURES SHOULD BE HANDLED WITH APPROPRIATE SAFETY PRECAUTIONS NECESSARY FOR THE HANDLING OF BLOOD BORNE PATHOGENS. PERSONNEL MUST BE TRAINED IN ACCORDANCE WITH THEIR INSTITUTIONAL POLICY REGARDING BLOOD BORNE PATHOGENS.

#### Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The text is available online at [www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

#### MR4 Replacement Policy

MR4 shall replace reagent if the customer reports it was received damaged. Shipments with problems must be reported within 30 days of receipt. Frozen shipments received thawed or damaged should be reported by the customer to the airline or freight forwarder upon receipt. MR4 should be notified after a claim has been filed to arrange for another shipment.

#### Disclaimers



# Product Information Sheet for MRA-593

## PARASITE

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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### Citations regarding use of this material

**Please remember to reference both MR4 AND THE DEPOSITOR in all publications resulting from the use of this reagent.**

### Example of how to reference MR4 reagents:

In Materials and Methods "*P. falciparum* line Dd2 (MRA-156, MR4, ATCC® Manassas Virginia)...". In the acknowledgment portion: "We thank MR4 for providing us with malaria parasites contributed by (name of depositor)."

### Consider Depositing to the MR4!

The generosity of other researchers made it possible for you to use this reagent. We invite you to share your reagents with the malaria community. One of the missions of MR4 is to facilitate technology transfer. MR4 will acknowledge your contribution in its publications. Contact us for more information.

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