



Product Information Sheet for MRA-1031

PARASITE

MR4 Number: MRA-1031

Organism: *Plasmodium falciparum*

Strain: *piggyBac* clone 0702090102H03

Alt. Strain designation: PB-116

Unit size: 0.5ml

Original Preparation Contributed By: John Adams

References:

Bharath Balu, Chitra Chauhan, Steven P Maher, Douglas A Shoue, Jessica C Kissinger, Malcolm J Fraser Jr and John H Adams. *piggyBac* is an effective tool for functional analysis of the *Plasmodium falciparum* genome. BMC Microbiology 2009, 9:83

Balu B, et al. High-efficiency transformation of *Plasmodium falciparum* by the lepidopteran transposable element *piggyBac*. Proc Natl Acad Sci U S A. 102: 16391-16396, 2005. PubMed: 16260745

Balu B, et al. A genetic screen for attenuated growth identifies genes crucial for intraerythrocytic development of *Plasmodium falciparum*. PLoS One. 5: e13282, 2010. PubMed: 20949012

Growth and Preservation Protocols

Recommended Blood Type for *in vitro* culture: Type O blood (washed); pooled human serum Type A or Type O recommended.

Growth Temperature: 37°C

Media Preparation: To make 1.0 L of **incomplete medium**, start with either 928 ml liquid RPMI-1640 (without NaHCO₃, without L-glutamine), or, to 900 ml Tissue Culture grade water, add 10.43 g of powdered RPMI-1640 (without NaHCO₃, without L-glutamine). To the RPMI-1640 media, add 25 ml of 1 M HEPES (final = 25 mM), 27 ml 7.5% sodium bicarbonate solution (final = 0.2% NaHCO₃), 10 ml of 200 mM L-glutamine (final = 2 mM), 10 ml 20% Glucose (final = 20 mM, optional), 27.2 mg hypoxanthine (10 ml of 20 mM hypoxanthine stock in 1M NaOH), 0.25 ml 10mg/ml Gentamicin (final = 2.5 ug/ml, optional). Add TC grade water to 1.0 L. Mix thoroughly and filter with 0.22 cm sterile filter unit. Store at 4°C. Incomplete media can be used for many applications involving wash steps during preparation of parasites for culture or assay. For 500 ml **complete medium**, add 50 ml of appropriate heat inactivated human serum (MR4: type A is used with washed type O blood) to 450 ml incomplete medium (final = 10% serum). Store at 4°C. If necessary, filter the complete media with 0.22 cm sterile filter unit and store at 4°C. Sera tend to clog the filter unit so use a pre-filter or improved filter unit able to handle human serum. (Note: complete media has a short shelf life; use within 7-14 days). To prepare heat inactivated sera, incubate sera for 45 min at 56°C, aliquot and store frozen at -20°C. Serum substitutes may be acceptable for growth of some, but not all, parasite strains, and may significantly impact virulence and parasite protein expression profiles.

Establishing a Culture from a Frozen Vial:

1. Thaw vial in 37° C water bath just until culture is completely thawed, transfer to hood, wipe outside surface of vial with 70% EtOH, transfer contents with 1 ml sterile pipette to sterile 50 ml conical centrifuge tube.

2. Add 12% sodium chloride (NaCl) solution dropwise, approximately 1:5 ratio NaCl to cell mixture (0.2x of original culture volume). Allow to stand for 5 min.
3. Using a 1ml syringe and 27G needle, add 10 volumes (original culture volume) of a 1.6% NaCl solution dropwise with shaking (10:1 ratio NaCl to original culture volume).
4. Centrifuge at 1000 x g for 5 minutes, and aspirate most of the solution leaving about 0.5-1 ml to resuspended the cells.
5. Resuspend the cells very gently by swirling the tube until most of the pellet is resuspended.
6. Add dropwise while shaking 10 volumes of complete media.
7. Centrifuge at 1000 x g for 5 minutes and aspirate the media.
8. Add 5 ml of complete medium, move the sample to 25cm² tissue culture flask.
9. For continuous culture add uninfected, washed RBCs to 1-2% hematocrit (immediately or the next day).
10. Gently outgas culture flask with gas mixture of 5% CO₂, 5% O₂, 90% N₂ through sterile, cotton plugged Pasteur pipet. (Alternatively consult protocols for candle jar incubation methods).
11. Take a smear for Giemsa staining after 24 hrs to evaluate parasite growth and determine parasitemia.

Daily Culture Maintenance and Blood Smear:

Changing of the culture medium every 24 hours is required for malaria-infected erythrocyte cultures.

1. Remove flask with infected culture from 37°C incubator and place flask onto flask warmer in the biological safety hood.
2. Carefully aspirate the medium with a sterile unplugged Pasteur pipet attached to the vacuum line. Remove as much fluid as possible without taking the cells.
3. Add 25 ml of sterile warm (37°C) complete medium to the flask, gently mix and aerate then quickly tighten cap of the flask and place the flask in the 37°C incubator till the next change.
4. To make a blood film: working in the biological safety hood, aspirate 0.5 –1 ml of mixed culture with a sterile pipet and put into an eppendorf tube.
5. Centrifuge the eppendorf tube at high speed and aspirate the supernatant. Mix the pellet and put 6 ul of the suspension on a glass slide for thick film smear or 2 ul for thin film smear. Spread the drop into a thin film with the edge of a clean glass slide. Air dry for 3 min at room temperature. Fix blood smear by rinsing it with methyl alcohol. Air dry for 3 min at room temperature. Stain blood films in 5% Giemsa solution for 15 min. Rinse with distilled water, air dry.
6. Using light microscopy at 100X magnification determine parasitemia of culture.

Cryopreservation:

Only immature parasite stage (rings) are viable by this method. We recommend a parasitemia of 3% or higher of ring stage parasites for cryopreservation.

1. Centrifuge the culture at 1000 x g for 5 min.
2. Wash the pellet once with 10 or more volumes of incomplete RPMI –1640 media. Centrifuge at 1800 for 5 min and leave sufficient supernatant to resuspend the pellet.
3. To the volume of packed RBCs, add slowly dropwise one volume of cold (4°C) Glycerolyte 57. Let stand for 5 min at room temperature.
4. Add an additional 3 volumes of cold Glycerolyte 57 to the pellet dropwise. Mix well and aliquot 0.5 ml into 1.5 ml sterile cryopreservation vials.
5. Place the samples into freezing containers (e.g., Nalgene Cryo 1° C / min Freezing Container) and store at –80°C for 24-48 hr.



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6. Transfer to liquid nitrogen for long term storage.

Important notes:

This reagent was authenticated by the contributor.
Please contact malaria@atcc.org for any comment.

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Biosafety Level: 2

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.

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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)."

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