Product Information Sheet for MRA-926

PARASITE

MR4 Number: MRA-926

Organism: *Plasmodium falciparum*

Strain: 7G8

Unit size: 0.5ml

Origin or parental strain: IMTM22

Parental Origin: Brazil (Manaus)

Date of cloning: 1984

Phenotype: Gametocyte producer- Close to parent of the cross. Minimised time in culture to preserve gametogenesis is recommended. Can be switched to Albumax for asexual growth.

Comments: Parental line for 7G8 x GB4 genetic cross progeny. Cross progeny clones available as MRA-932 through MRA-966. Self fertilized progeny clones available as MRA-927 through MRA-931.

Drug Profile: Chloroquine-RESISTANT, Pyrimethamine-RESISTANT

Cloner: Thomas Burkot

Depositor: Thomas Wellems

References:

Burkot, T., Williams, J.L., Schneir, I. 1984. Infectivity to mosquitoes of Plasmodium falciparum clones grown in vitro from the same isolate. Trans. R. Soc. Trop. Med. Hyg., 78, 339-341

Hayton, K.,Gaur G., Takahashi, J., Henschen, B., Lambert, L. Furuya, T., Bouttenot, R., Doll, m., Nawaz, N., jiang L., L. H and Wellems, T.E. 2008. Erythrocyte Binding Protein PfRH5 Polymorphisms Determine Species-Specific Pathways of Plasmodium *falciparum* invasion. Cell Host & Microbe ,4, 40-51

Blood used: 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 $NaHCO_3$, without L-glutamine), or, to 900 ml Tissue Culture grade water, add 10.43 g of powdered RPMI-1640 (without $NaHCO_3$, 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% $NaHCO_3$), 10 ml of 200 mM L-glutamine (final =2 mM), 10 ml 20% Glucose (final = 20 mM, optional), 50 ug hypoxanthine (10 ml of 0.5 ug/ml hypoxanthine; final =0.005 ug/ml, optional), 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; usually 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 aerate culture with gas mixture of 5% CO_2, 5%O_2, 90% N_2 through sterile, cotton plugged Pasteur pipet.
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 cabinet, 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.

MR4/American Type Culture Collection (ATCC®)
10801 University Blvd.
Manassas, VA 20110-2209

Ordering info: 800-638-6597 (USA and Canada)
703-365-2765 (elsewhere); Fax: 703-365-2774
E-mail: malaria@atcc.org; web site: www.malaria.mr4.org

Product Information Sheet for MRA-926

PARASITE

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.
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.

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: 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.

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

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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.

MR4/American Type Culture Collection (ATCC®)
10801 University Blvd.
Manassas, VA 20110-2209

Ordering info: 800-638-6597 (USA and Canada)
703-365-2765 (elsewhere); Fax: 703-365-2774
E-mail: malaria@atcc.org; web site: www.malaria.mr4.org