Product Information Sheet for MRA-843

Plasmodium falciparum, Strain Dd2^{attB}

Catalog No. MRA-843

For research use only. Not for human use.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Plasmodiidae, Plasmodium*
Species: *Plasmodium falciparum*
Strain: Dd2^{attB}
Original Source: *Plasmodium falciparum* (*P. falciparum*), strain Dd2^{attB} was generated by integration of the acceptor *attB* site, recognized by the mycobacteriophage Bxb1 integrase during site-specific integration, into the nonessential glutaredoxin-like *cg6* gene located on chromosome 7.[1-3] *P. falciparum*, strain Dd2 originated in 1980 in Indochina.[4]
Comment: The whole genome sequence of *P. falciparum*, strain Dd2 is available (GenBank: AASM00000000).

The *P. falciparum* Dd2^{attB} parasite harbors an *attB* site at the *cg6* locus on chromosome 7, providing a site for integrase-mediated *attB* × *attP* integration. The targeting vector is pCG6-attB (available as BEI Resources MRA-844). Dual transfection *attP* and Bxb1 integrase encoding plasmids are available (BEI Resources MRA-846 and MRA-847) for single-copy plasmid integration into this host.

Material Provided:

Each vial of MRA-843 contains approximately 0.5 mL of *P. falciparum* infected human blood in Glycerolyte 57 solution (1:5). Please see Appendix I for cryopreservation instructions.

Packaging/Storage:

MRA-843 was packaged aseptically in cryovials. The product is provided frozen and should be stored at -80°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended (-130°C or colder). Freeze-thaw cycles should be avoided.

Growth Conditions:

RPMI 1640 medium, adjusted to contain 10% (v/v) heat-inactivated human serum, 25 mM HEPES, 2 mM L-glutamine
Human serum (pooled Type A or Type O recommended)
Please see Appendix II for complete medium preparation instructions and notes.

Incubation:
Temperature: 37°C
Atmosphere: 90% N_2, 5% CO_2, 5% O_2
Propagation:

1. Place the frozen vial in a 37°C water bath until the culture is completely thawed. Transfer the vial to a biological safety hood and wipe the outside surface of the vial with 70% ethanol.
2. Using a sterile 1 mL pipette, aseptically transfer the contents of the vial to a sterile 50 mL conical centrifuge tube.
3. Add 12% sodium chloride (NaCl) solution dropwise, approximately 1:5 ratio NaCl to cell mixture (0.2× original culture volume). Allow to stand for 5 minutes.
4. Using a 1 mL syringe and 27-gauge needle, add dropwise while shaking 10 volumes of a 1.6% NaCl solution (10:1 ratio NaCl to original culture volume).
5. Centrifuge at 1000 × g for 5 minutes and remove most of the supernatant, leaving approximately 0.5 mL to 1 mL to resuspend the cell pellet. Resuspend the cells by gently swirling the tube.
6. Add dropwise while shaking 10 volumes of complete medium. Centrifuge at 1000 × g for 5 minutes and carefully remove the supernatant.
7. Add 5 mL of complete medium and transfer the sample to a 25 cm² tissue culture flask.
8. For continuous culture, add uninfected red blood cells (RBCs) to a 1% to 2% hematocrit solution (immediately or the next day).
9. Gently aerate culture with a 95% air, 5% CO_2 gas mixture through a sterile, cotton-plugged Pasteur pipet. Incubate the flask at 37°C.
10. Take a smear for Giemsa staining after 24 hours to evaluate parasite growth and determine parasitemia.

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

1. Remove the flask with infected culture from the 37°C incubator and place onto a flask warmer.
2. Carefully remove the supernatant with a sterile, unplugged Pasteur pipet under vacuum. Remove as much of the supernatant 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 the cap and place the flask in the 37°C incubator until the next change of medium.

Preparation of Blood Smear:

1. Carefully remove 0.5 mL to 1 mL of mixed culture with a sterile pipet and transfer to a microcentrifuge tube.
2. Centrifuge the microcentrifuge tube at high speed and aspirate the supernatant.
3. Mix the pellet and transfer 6 µL of the suspension to a glass slide for a thick film smear or 2 µL for a thin film smear. Spread the drop into a thin film using the edge of a clean glass slide. Air dry for 3 minutes at room temperature.
4. Fix the blood smear by rinsing it with methyl alcohol. Air

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dry for 3 minutes at room temperature.
5. Stain blood films in 5% Giemsa solution for 15 minutes. Rinse with distilled water and allow to air dry.
6. Using light microscopy at 100× magnification, determine parasitemia of culture.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Plasmodium falciparum*, Strain Dd2^{attb}, MRA-843, contributed by David A. Fidock."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

All blood cultures should be handled with appropriate safety precautions necessary for the handling of bloodborne pathogens. Personnel must be trained in accordance with their institutional policy regarding bloodborne pathogens.

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References:

1. Nkrumah, L. J., et al. "Efficient Site-Specific Integration in *Plasmodium falciparum* Chromosomes Mediated by Mycobacteriophage Bxb1 Integrase." Nat. Methods 3 (2006): 615-621. PubMed: 16862136.
2. Adjalley, S. H., M. C. Lee, and D. A. Fidock. "A Method for Rapid Genetic Integration into *Plasmodium falciparum* Utilizing Mycobacteriophage Bxb1 Integrase." Methods Mol. Biol. 634 (2010): 87-100. PubMed: 20676977.
3. Fidock, D. A., Personal Communication.
4. Wootton, J. C., et al. "Genetic Diversity and Chloroquine Selective Sweeps in *Plasmodium falciparum*." Nature 418 (2002): 320-323. PubMed: 12124623.

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APPENDIX I: CRYOPRESERVATION

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

1. Centrifuge the culture at 1000 × g for 5 minutes.
2. Wash the pellet once with 10 or more volumes of incomplete RPMI 1640 medium. Centrifuge at 1800 × g for 5 minutes and leave sufficient supernatant to resuspend the pellet.
3. To the volume of packed red blood cells, slowly add dropwise one volume of cold (4°C) Glycerolyte 57 solution. Let stand for 5 minutes at room temperature.
4. Add dropwise an additional 3 volumes of cold Glycerolyte 57 solution to the pellet. Mix well and aliquot 0.5 mL into 1.5 mL sterile cryopreservation vials.
5. Place the vials in a controlled-rate freezing unit. From room temperature, cool the vials at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 24 to 48 hours and then plunge vials into liquid nitrogen.
6. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

APPENDIX II: MEDIA PREPARATION

1. Incomplete Medium: used for many applications involving wash steps during preparation of parasites for culture or assay. The incomplete medium consists of RPMI 1640 medium supplemented with the following components[1]:

Incomplete Medium	
RPMI 1640 medium[2,3]	
Sodium bicarbonate ($NaHCO_3$)[4]	2.0 g/L
L-Glutamine	2 mM
HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]	25 mM
Optional:	
D-Glucose[5]	2.0 g/L
Hypoxanthine	5 µg/L
Gentamycin	2.5 mg/L

[1]Prepare sterile stock solutions at concentrations that are easily diluted into the liquid medium to obtain the appropriate user concentrations, and add aseptically. Ready-made stock solutions for many of the components are available from numerous manufacturers.
[2]RPMI 1640 medium is available from numerous manufacturers as both a powder and a sterile, prepared liquid, with or without L-glutamine and HEPES. If using powdered RPMI 1640 medium, prepare the medium following manufacturer instructions, sterile-filter using a 0.22 µm filter, then aseptically add the necessary components in the appropriate concentrations.
[3]If stock solutions were not sterile or aseptic techniques were not followed, sterile-filter the medium using a 0.22 µm filter after the addition of all components. Store at 4°C.
[4]Prepared, liquid medium typically contains sodium bicarbonate while powdered medium does not. A typical concentration of sodium bicarbonate in RPMI 1640 medium is 2 g/L, though some formulations contain different amounts.
[5]A typical concentration of D-glucose in RPMI 1640 medium is 2 g/L. The option to supplement with an additional 2 g/L yields a final concentration of 4 g/L D-glucose.

2. Complete Medium: consists of incomplete medium (above) supplemented with 10% heat-inactivated human serum. If necessary, filter the complete medium with a 0.22 µm filter. Since serum tends to clog sterilizing filters, a serum pre-filter may be used first, followed by a 0.22 µm sterilizing filter.

   Note: Human serum type A is used with washed type O blood. Serum substitutes may be used, however they may not be acceptable for all parasite strains.

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