Product Information Sheet for MRA-1318

Plasmodium falciparum, Strain MRA1240-hap2

Catalog No. MRA-1318

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Contributor and Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Plasmodiidae*, *Plasmodium*
Species: *Plasmodium falciparum*
Strain: MRA1240-hap2
Original Source: *Plasmodium falciparum (P. falciparum)*, strain MRA1240-hap2 is a haplotype-specific drug response phenotype cloned from the multiclonal strain *P. falciparum*, strain IPC 5202 (BEI Resources MRA-1240), which was originally isolated in 2011 from a human patient with malaria in Battambang Province, western Cambodia.[1,2] Strain IPC 5202 was cloned by limiting dilution and the resulting clones were genotyped at 24 highly polymorphic single nucleotide polymorphisms to determine individual haplotypes.[1]
Comments: *P. falciparum*, strain MRA1240-hap2 is resistant to mefloquine and exhibited a drug susceptibility profile with IC_{50} values of 78.7 ± 7.3 (moderately resistant) for chloroquine, 42.4 ± 2.9 (resistant) for mefloquine and 35.2 ± 4.1 (sensitive) for piperaquine.[1]

Material Provided:

Each vial of MRA-1318 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-1318 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 (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 2 grams per liter D-glucose, 27 µg per mL hypoxanthine and 5 µg per mL gentamicin (optional)
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 90% N_2, 5% CO_2, 5% O_2 gas mixture through a sterile, cotton-plugged Pasteur pipet. Incubate the flask at 37°C.
10. Take a smear for Giemsa staining after 1 day to evaluate parasite growth and determine parasitemia.

Maintenance:
Note: Changing of the culture medium every 1 day 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 dry for 3 minutes at room temperature.
5. Stain blood films in 10% 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 MRA1240-hap2, MRA-1318, contributed by BEI Resources."

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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: 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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; 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. Nkhoma, S. C., et al. "Dissection of Haplotype-Specific Drug Response Phenotypes in Multiclonal Malaria Isolates." Int. J. Parasitol. Drugs Drug Resist. 15 (2021): 152-161. PubMed: 33780700.
2. Ariey, F., et al. "A Molecular Marker of Artemisinin-Resistant *Plasmodium falciparum* Malaria." Nature 505 (2014): 50-55. PubMed: 24352242.

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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 4 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 per minute to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C per minute 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 1 to 2 days 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]	2.4 g per L
L-Glutamine	2 mM
HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]	25 mM
D-Glucose[5]	2 g per L
Hypoxanthine	27 µg per mL
Gentamicin (optional)	5 µg per mL

[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 grams per liter, though some formulations contain different amounts.
[5]A typical concentration of D-glucose in RPMI 1640 medium is 2 grams per liter. The option to supplement with an additional 2 grams per liter yields a final concentration of 4 grams per liter 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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