Product Information Sheet for MRA-1332

Plasmodium falciparum, Strain LA476-1Δ*hrp*2/Δ*hrp*3

Catalog No. MRA-1332

For research use only. Not for use in humans.

Contributor:
Tim Anderson, Professor, Department of Disease Intervention and Prevention, Texas Biomedical Research Institute, San Antonio, Texas, USA

Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Plasmodiidae*, *Plasmodium*
Species: *Plasmodium falciparum*
Strain: LA476-1Δ*hrp*2/Δ*hrp*3
Original Source: *Plasmodium falciparum (P. falciparum),* strain LA476-1Δ*hrp*2/Δ*hrp*3 is deletion mutant derived from the progenitor strain LA476-1 (MRA-1330).[1,2] Strain LA476-1 is a clone of *P. falciparum*, strain LA476, which was originally isolated in 2008 from a patient in Malawi.[1,2,3]
Comments: Strain LA476-1Δ*hrp*2/Δ*hrp*3 was generated by the deletion of the *P. falciparum* histidine-rich protein 2 (*hrp*2) and 3 (*hrp*3) genes, located outside of the telomeric region of chromosomes 8 and 13.2, respectively, using CRISPR-Cas9 technology.[1,2]

Rapid diagnostic tests (RDTs) based on the malaria histidine-rich proteins (HRP) 2 and 3 play a key role in malaria diagnosis and have replaced microscopy as the laboratory confirmatory tool for clinical malaria diagnoses in most malaria-endemic countries.[4] However, natural deletions of HRP2- and HRP3-encoding genes, *hrp*2 and *hrp*2, respectively, can undermine the practical utility of these RDTs. Therefore, deletion mutants are valuable tools to study the evolution and spread of HRP deletions in natural parasite populations.[2]

Material Provided:
Each vial of MRA-1332 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-1332 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 (Type A), 25 mM HEPES, 2 mM L-glutamine, 2 g/L D-glucose, 27 µg/mL hypoxanthine and 5 µg/mL gentamicin (optional)
Human serum (pooled Type A or Type O recommended)[1]
Please see Appendix II for complete medium preparation instructions.
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 it to stand for 5 minutes.
4. Using a 1 mL syringe equipped with a 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 and 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 it 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 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 LA476-1Δ*hrp*2/Δ*hrp*3, MRA-1332, contributed by Tim Anderson."

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 (BMBL). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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. Anderson, P., Personal Communication.
2. Nair, S., et al. "Fitness Costs of *pfhrp*2 and *pfhrp*3 Deletions Underlying Diagnostic Evasion in Malaria Parasites." J. Infect. Dis. 226 (2022): 1637-1645. PubMed: 35709327.
3. Nkhoma S. C., et al. "Close Kinship within Multiple-Genotype Malaria Parasite Infections." Proc. Biol. Sci. 279 (2012): 2589-2598. PubMed: 22398165.
4. Beshir, K. B., et al. "Screening Strategies and Laboratory Assays to Support *Plasmodium falciparum* Histidine-Rich Protein Deletion Surveillance: Where We Are and What is Needed." Malar. J. 21 (2022): PubMed: 35751070.

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