Product Information Sheet for MRA-1185

PARASITE

MR4 Number: MRA-1185
Organism: Plasmodium falciparum
Strain: SenTh029.09
Alt. Strain designation: T29.09
Geographic Origin: Human patient isolate, Thiès, Senegal
Date of isolation: 2009
Genotype: CACTCGAGATTGCCCCTACGCCTG
(Broad 24 SNP Bar Code, Daniels et al. Malaria Journal 7:223, 2008)
Unit size: 0.5ml
Depositor: Dyann Wirth, Sarah Volkman, Harvard School of Public Health; Souleymane Mboup, Daouda Ndiaye

References:

MR4 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 298 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 room temperature.

To make 1.0 L of 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. 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. Centrifuge the culture at 1000 x g for 5 min. RPMI –1640 media. Centrifuge at 1800 for 5 min and leave sufficient 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. 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) Glycerol 57. Let stand for 5 min at room temperature.
4. Add an additional 3 volumes of cold Glycerol 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.
Parasite

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

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Citations regarding use of this material
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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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