PARASITE

MR4 Number: MRA-1191
Organism: Plasmodium falciparum
Strain: SenTh105.07
Alt. Strain designation: T105.07
Geographic Origin: Human patient isolate, Thiès, Senegal
Date of isolation: 2007
Genotype: CACTCGAGATCXXCAATGATTG
(Broad 24 SNP Bar Code, Daniels et al.
Malaria Journal 7:223, 2008)

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 928 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 = 0.2% NaHCO₃), 10 ml of 200 mM L-glutamine, 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 4°C.

To make 1.0 L of complete medium, start with either 928 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 4°C.

Intracytoplasmic Hematocrit: 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; use within 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, gently mix and aerate then quickly tighten cap of the flask and place the flask in the 37°C incubator till the next change.

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.

BEI Resources/MR4
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Biosafety Level: 2

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