Product Information Sheet for MRA-975

HC-04, Hepatocyte (human)

Catalog No. MRA-975

For research use only. Not for human use.

Contributor:
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Manufacturer:
BEI Resources

Product Description:
MRA-975 is a hepatocyte line derived from normal human liver cells which supports *Plasmodium falciparum* (*P. falciparum*) and *P. vivax* liver stage development.[1]

According to results from two separately and independently performed assays testing for genetic profiles, STR (Short-tandem-repeats) and HLA-class I molecules, HC-04 appears nearly identical to the HepG-2 cell line. However, analysis of functional properties associated with each of the cell lines demonstrated that HC-04 is able to support growth and development of *P. falciparum* sporozoites to liver/blood stage parasites, while the HepG-2 cell line is not. Therefore, the two cell lines function in a different and unique manner in regards to *P. falciparum* sporozoite infection/development, despite genetic similarity.

Karyotypic analysis performed by the contributor indicated that the cells are all in the hyperdiploid range (2n = 48-50).[1] Abnormal chromosomes include a chromosome 1p deletion, a chromosome 6 derivative, triplet of chromosome 7, and a chromosome 15 derivative. STR analysis from both passage 8 and passage 56 cells provided by two independent laboratories at the WRAIR in 2009-2010 produced identical STR results shown below (identical results were also obtained by BEI Resources).

Amelogenin	X,Y
CSF1PO	10,11
D13S317	9,13
D16S539	12
D5S818	11,12
D7S820	10
THO1	9
TPOX	8,9
Vwa	17

Material Provided:
Each vial of MRA-975 contains approximately 0.5 mL of HC-04 cells in complete culture medium (CCM) supplemented with 10% fetal bovine serum (FBS) and 10% dimethylsulfoxide (DMSO). Please see Appendix I for media preparation. Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as cells per vial, is shown on individual certificates of analysis for each product lot.

Packaging/Storage:
This product was packaged aseptically, in screw-capped plastic cryovials. It should be stored at cryogenic temperature (-100°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. Storage at -70°C will result in loss of viability. To insure the highest level of viability, the vial should be thawed and the culture initiated as soon as possible upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product after thawing. For transfer between freezers and shipping, the cells may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to reconstituting this material.

Safety Precautions:
When handling frozen vials it is highly recommended that protective gloves, lab coat and full face mask be worn. Even brief exposure to the ultra-cold temperature can cause tissue damage from frostbite. Also, some vials may slowly fill with liquid nitrogen if they have been immersed during cryogenic storage. When thawing, the liquid nitrogen may rapidly expand as it changes to gas, breaking the vial or cap with explosive force, sending debris flying with enough velocity to cause injury. Store and use in areas with adequate ventilation.

Growth Conditions:
Prior to thawing the cells, prepare CCM according to Appendix I. Thaw 1 vial in a 37°C water bath and transfer the contents into a 75 cm cell culture flask with 18 mL of CCM. Keep the flask tightly capped in a 37°C incubator. Change the media at 12 to 16 hours post seeding. Feed the cells at least every 48 hours, harvest at 80% confluency and reseed at a 1:4 to 1:5 ratio.

Sub-culture procedure: Trypsin or trypsin-like enzyme substitute may be used to fully disperse adherent cells. Remove complete medium from flask and wash cell monolayer with equal volume sterile PBS. Aspirate PBS, add minimal volume of enzyme buffer sufficient to coat cell monolayer. Following incubation at 37°C for 5 to 7 minutes, knock flask to encourage dispersion of cell monolayer. Expose cell monolayer to enzyme only long enough to disrupt cell contacts. Return to complete medium immediately following dispersion, determine cell count as required and passage to new flasks.

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Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: HC-04, Hepatocyte (human), MRA-975, contributed by Jetsumon Sattabongkot Prachumsri."

Biosafety Level: 1
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.

Disclaimers:
You are authorized to use this product for research use only. It is not intended for human use.

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MRA-975 is claimed in U.S. Patent Number 7,015,036 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof. Any commercial use will require specific written permission from the contributor's institution.

References:
1. Sattabongkot, J., et al. "Establishment of a Human Hepatocyte Line that Supports *in vitro* Development of the Exo-Erythrocytic Stages of the Malaria Parasites *Plasmodium falciparum* and *P. vivax*." Am. J. Trop. Med. Hyg. 74 (2006): 708-715. PubMed: 16687667.
2. Cui, L., et al. "Culture of Exoerythrocytic Stages of the Malaria Parasites *Plasmodium falciparum* and *Plasmodium vivax*." Methods Mol. Biol. 470 (2009): 263-273. PubMed: 19089388.
3. VanBuskirk, K. M., et al. "Preerythrocytic, Live-Attenuated *Plasmodium falciparum* Vaccine Candidates by Design." Proc. Natl. Acad. Sci. USA 106 (2009): 13004-13009. PubMed: 19625622.
4. Mikolajczak, S. A., et al. "Disruption of the *Plasmodium falciparum* Liver-Stage Antigen-1 Locus Causes a Differentiation Defect in Late Liver-Stage Parasites." Cell Microbiol. 13 (2011): 1250-1260. PubMed: 21569184.

APPENDIX I: MEDIA PREPARATION

HC-04 Complete Culture Medium (CCM; 1 L)

Modified Eagle's Medium (MEM; - L-glu)	428.75 mL
F-12 Nutrient Mixture (+ L-glu)	428.75 mL
HEPES (1 M stock; 15 mM final)	15 mL
Sodium bicarbonate (NaHCO3; 7.5% stock; 1.5 g/L final)	20 mL
L-glutamine (200 mM stock; 2.5 mM final)	7.5 mL
FBS, culture tested (10% final)	100 mL

Freezing Medium
- HC-04 CCM
- 10% FBS
- 10% DMSO

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