Human Cell Line, SMC5 Knockout CEM-SS

Catalog No. HRP-20251

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

NIH HIV Reagent Program

Product Description:

HRP-20251 is a CEM-SS cell (human CD4 T-cell) line with the SMC5 component knocked out of the Structural Maintenance of Chromosome (SMC) 5/6 complex.[1] It is a suspension cell line. Recent studies support that the SMC5/6 complex plays a direct role in mediating the establishment of human immunodeficiency virus type 1 (HIV-1) latency by epigenetically silencing integration-competent HIV-1 proviruses before integration.[1]

Material Provided:

Each vial contains approximately 1.0 mL of frozen cells in 90% fetal bovine serum and 10% dimethylsulfoxide (DMSO).

Packaging/Storage:

This product was packaged aseptically, in screw-capped plastic cryovials. It should be stored at -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 ensure 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 the transfer between freezers and shipping, the cells may be placed on dry ice for brief periods, although the 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.

Thawing and Growth:

Note: CEM-SS is a suspension cell line. Do not discard floating cells.

Prior to thawing the SMC5 knockout CEM-SS cells, prepare growth medium (GM) for use. SMC5 knockout CEM-SS cells are grown in RPMI-1640 medium (ATCC® 30-2001™) supplemented with 10% fetal bovine serum (ATCC® 30-2020™) and, after initial culture, the cells are maintained by adding 1 µg/mL puromycin (Gibco™). Start cells in media without selection antibiotic (puromycin) for several days, then replace with media with puromycin for the remainder of growth. These GMs are formulated for use with a 5% CO_2 in air atmosphere.

Rapidly thaw the vial of cells in a 37°C water bath with gentle agitation. To reduce the risk of contamination, keep the cap and O-ring of the vial out of the water and repeatedly check the cap for tightness during thawing. Remove from the water bath immediately when thawed. Dry the vial with a sterile wiper, decontaminate using a wiper soaked with 70% isopropyl alcohol, and let the vial air dry. Aseptically open the vial, remove the vial contents and add to 4.0 mL of GM in a centrifuge tube. Centrifuge the cell suspension at 150 × g for 8 to 10 minutes at 18°C to 25°C. Discard the supernatant and resuspend the cell pellet in 10 mL of pre-warmed GM without puromycin. Transfer the cell suspension into a 75 cm^2 tissue culture flask. Incubate the new culture at 37°C and 5% CO_2.

After 1 to 2 days, perform a total media change by placing the flask contents into a centrifuge tube. Centrifuge the cell suspension at 150 × g for 8 to 10 minutes at 18°C to 25°C. Discard the supernatant and resuspend the cell pellet in 10 mL of pre-warmed GM (containing puromycin).

Sub-culture procedure.
Cultures can be maintained by the addition of fresh GM or the replacement of GM. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1.0×10^5 cells/mL. Maintain a cell density between 1.0×10^5 and 1.0×10^6 cells/mL. To replace the GM, centrifuge the cell suspension at 150 × g to 300 × g for 8 to 10 minutes at 18°C to 25°C, remove the supernatant and resuspend the cell pellet in fresh pre-warmed complete GM to the desired cell density. Add fresh medium every 2 to 3 days, depending on cell density.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH HIV Reagent Program, NIAID, NIH: Human Cell Line, SMC5 Knockout CEM-SS, HRP-20251, contributed by Dr. Bryan R. Cullen."

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.

Disclaimers:

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

Use of this product is subject to the terms and conditions of the NIH HIV Reagent Program Material Transfer Agreement (MTA). The MTA is available on our Web site at www.hivreagentprogram.org.

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References:

1. Irwan, I. D., H. P. Bogerd and B. R. Cullen. “Epigenetic Silencing by the SMC5/6 Complex Mediates HIV-1 Latency.” Nat. Microbiol. 12 (2022): 2101-2113. PubMed: 36376394.

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