iTIME.219, Inducible Telomerase-Immortalized Endothelial Cells Infected with Recombinant Kaposi's Sarcoma-Related Herpesvirus

Catalog No. NR-56694

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

iTIME.219 is an inducible telomerase-immortalized endothelial (iTIME) cell line infected with Kaposi's sarcoma-associated herpesvirus (KSHV) engineered to maintain the recombinant reporter virus rKSHV.219 in the latent phase and transition to lytic replication and infectious virus release upon induction by a KSHV-specific stimulus. Latent-phase rKSHV.219 is measurable through constitutive expression of enhanced green fluorescent protein (eGFP). Activation of lytic-phase rKSHV.219 is signaled through expression of red fluorescent protein (RFP). rKSHV.219 contains the puromycin-resistance gene linked to the constitutive Rouse sarcoma virus promoter, which enables positive selection of cells harboring the KSHV episome. When exposed to either sodium butyrate or doxycycline, the cells are activated to lytic replication as evidenced by the expression of RFP and KSHV lytic genes and release of large quantities of infectious virus.[1,2]

iTIME219 was produced by transduction of rKSHV.219-infected TIME cells with a plasmid containing the viral replication and transcription activator (RTA) gene under the control of a doxycycline-inducible system.[1,2]

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in non-selective cell growth medium (CGM) (90%) and dimethylsulfoxide (DMSO; 10%) cryopreservative. 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 lot.

Packaging/Storage:

NR-56694 was packaged aseptically in screw-capped plastic cryovials. The product 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.

Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

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 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.

Thawing and Growth:

Prior to thawing the cells, prepare non-selective and selective cell growth media (CGM) for use (refer to Appendix I and II). The CGMs are formulated for use in an aerobic atmosphere with 5% CO_2.

Rapidly thaw the vial of cells in a 37°C water bath. 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 wipe, 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 a centrifuge tube containing 4 mL of non-selective CGM. Centrifuge the cell suspension at 125 × 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 **non-selective** CGM. Transfer the cell suspension into a 75 cm^2 tissue culture flask. Incubate the new culture at 37°C in an aerobic atmosphere with 5% CO_2. After 48 hours, remove the non-selective media and replace with an equal amount of selective CGM.

Sub-culture procedure:

After trypsinizing the monolayer using standard methods, aseptically transfer the contents of the flask to a centrifuge tube containing an equal volume of selective CGM. Centrifuge the cell suspension at 125 × g for 8 to 10 minutes at 18°C to 25°C. Discard the supernatant and resuspend the cell pellet in an equal amount of pre-warmed non-selective CGM as the original volume. Perform a cell count and add appropriate aliquots of the cell suspension to as many 75 cm^2 tissue culture flasks as needed to yield a sub-cultivation ratio of 1:3 to 1:4. Adjust the volume of GM to 15 mL to 20 mL for a 75 cm^2 flask. Incubate the new culture at 37°C in an aerobic atmosphere with 5% CO_2.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH:

iTIME.219, Inducible Telomerase-Immortalized Endothelial Cells Infected with Recombinant Kaposi's Sarcoma-Related Herpesvirus, NR-56694."

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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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References:

1. Berger, E., Personal Communication.
2. Dollery, S. J., et al. "iTIME.219: An Immortalized KSHV Infected Endothelial Cell Line Inducible by a KSHV-Specific Stimulus to Transition from Latency to Lytic Replication and Infection Virus Release." Front. Cell. Infect. Microbiol. 11 (2021): 654396. PubMed: 33937098.

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Appendix I: Non-Selective Cell Growth Medium

Prepare the Non-Selective CGM following the recipe below and filter-sterilize using a 0.2 µm filter.

VascuLife® Basal Medium (Lifeline® Cell Technology LM-0002)	475 mL
VascuLife®SMC LifeFactors Kit (Lifeline® Cell Technology LS-1040)	
rh FGF Basic LifeFactor (to final concentration of 5 ng per mL)	0.5 mL
rh Insulin LifeFactor (to final concentration of 5 µg per mL)	0.5 mL
Ascorbic acid LifeFactor (to final concentration of 50 µg per mL	0.5 mL
L-Glutamine LifeFactor (to final concentration of 10 mM)	25.0 mL
rh EGF LifeFactor (to final concentration of 5 ng per mL)	0.5 mL
FBS LifeFactor (to final concentration of 5%)	25.0 mL

Appendix II: Selective Cell Growth Medium

Prepare the selective CGM following the recipe below and filter-sterilize using a 0.2 µm filter.

Non-Selective CGM	526 mL
Blasticidin (to final concentration of 12.5 µg per mL)	0.66 mL
G418 Sulfate (to final concentration of 200 µg per mL)	2.1 mL
Puromycin dyhydrochloride (to final concentration of 10 µg per mL)	0.53 mL

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