

Certificate of Analysis for NR-56694

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

Catalog No. NR-56694

This reagent is the tangible property of the U.S. Government.

Product Description:

TIME.219 is an inducible telomerase-immortalized endothelial (iTIME) cell line infected with Kaposi's sarcomaassociated 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).

Lot: 70055217 Manufacturing Date: 11OCT2022

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TEST	SPECIFICATIONS	RESULTS
Growth Properties	Adherent	Adherent
Morphology	Endothelial	Endothelial
Multiplex PCR Amplification of Cytochrome C Oxidase I (COI) Gene	Human origin No evidence of another species	Human origin No evidence of another species
Short Tandem Repeat (STR) Analysis	Consistent with reference profile (TIME, ATCC® CRL-4025™)	Consistent with reference profile (TIME, ATCC® CRL-4025™)
Total Cell Count	> 1.0 × 10 ⁶ cells per vial	3.4 × 10 ⁶ cells per vial
Post-Freeze Viability	≥ 50%	50.2%
Phenotypic Analysis		
eGFP expression in latent-phase rKSHV.219	eGFP expression	eGFP expression (Figure 1)
RFP expression in lytic-phase rKSHV.219 ¹	RFP expression	RFP expression (Figure 2)
Sterility (21-day incubation)	·	, ,
Harpo's HTYE broth, 37°C and 26°C, aerobic ²	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO ₂	No growth	No growth
Mycoplasma Contamination		
Hoechst DNA stain	None detected	None detected
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

¹Activation of lytic phase rKSHV.219 through exposure to sodium butyrate or doxycycline. For more information, please refer to 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." <u>Front. Cell. Infect. Microbiol.</u> 11 (2021): 654396. PubMed: 33937098.)

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²Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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Figure 1: Expression of eGFP in Latent Phase

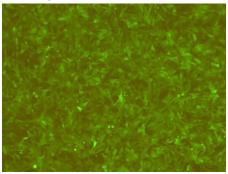
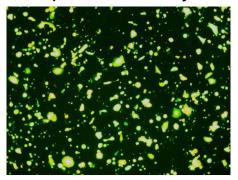


Figure 2: Expression of RFP in Lytic Phase



/Sonia Bjorum Brower/ Sonia Bjorum Brower

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