CERTIFICATE OF ANALYSIS

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Cas9 Stable Cell Line

Product Information	
Cell Line	A549
Parental (ATCC ID)	CCL-185
Catalog ID	HD Cas9-001
SNB ID	51424
Cas9 expression promotor	CAG
Passage	4
Cryopreservation Date	17/02/2022

Properties	
Total viable cells	1e6
Total Volume	1ml
Cryopreservation Medium	45%RPMI 1640, 50% AU FBS, 5%DMSO
Storage Conditions	Liquid nitrogen vapour phase

Customer Support	
Technical Support	technical@horizondiscovery.com
Customer Service	orders@horizondiscovery.com

Quality Control

Test	Test Method	Result
Viability	Post bank thawing and cultivation	N/A
Sterility	Direct inoculation of Tryptic Soy and Thioglycolate Broths	N/A
Mycoplasma	Mycoplasma detection by qPCR	N/A
Characterisation	Functionality confirmed by gene editing assay (> 20%)	N/A

Growth Conditions

Recommended Culture Medium	RPMI 1640, 10% FBS, 1% Pen/Strep
Cell Line Revival	Rapidly thaw cells in a 37°C water bathfor 2 minutesuntil nearly (80%) thawed. Transfer contents into a tube containing pre-warmed media. Centrifuge the cellsat 300 x gfor 4minutesand remove the supernatant.Add 2 mL of appropriate cell culture medium and transfer cells to T25 flask containing 4 mL of pre-warmed cell culture medium. Place cell in a humidified 37°C incubator with 5% CO2. Gently replace medium after 24 hours with 5-10 mL of appropriate cell culture medium and continue culturing at 37°Cwith 5% CO2.When appropriate(70-80% confluency), expand cell lines to a T75 flask using the subculturingprocedures below.
Subculture	Carefully aspirate the growth medium from the cells. Gently wash cells with 7.5 mL PBS to remove the remaining media. Trypsinize the cells with 3 mL trypsin-EDTA solution. Place the flask in the 37 °C incubator for approximately 2 minutes or until the cells release from the flask. Add 15-30 mL of the appropriate Cell Culture Medium to resuspend the detached cells and inactivate the trypsin. Pipette cells up and down ~ 5 times with a 10 mL pipette to obtain a single cell suspension, while avoiding frothing of medium. Plate cells into new sterile flasks or plates containing appropriate Cell Culture Medium. Place the cells in a humidified 37 °C incubator with 5% CO2.
Recommended Cryopreservation Medium	45% RPMI 1640, 50% FBS, 5% DMSO

Additional Information

For the full Technical Manual and protocols, please visit horizondiscovery.com

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