

Cas9 Stable Cell Line

Product Information	
Cell Line	RKO Cas9 Stable Cell Line
Parental (ATCC ID)	CRL-2577
Catalog ID	HD Cas9-007
SNB ID	42346
Cas9 expression promotor	hEF1α
Passage	11
Cryopreservation Date	February 27, 2020

Properties	
Total viable cells	> 1x10 ⁶
Total Volume	1 mL
Cryopreservation Medium	45% RPMI 1640, 50% 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	Pass
Sterility	Direct inoculation of Tryptic Soy and Thioglycolate Broths	Pass
Mycoplasma	Mycoplasma detection by qPCR	Pass
Characterisation	Functionality confirmed by gene editing assay (> 20%)	Pass

Growth Conditions		
Recommended Culture Medium	RPMI 1640, 10% FBS, 1% Pen/Strep	
Cell Line Revival	Rapidly thaw cells in a 37°C water bath for 2 minutes until nearly (80%) thawed. Transfer contents into a tube containing pre-warmed media. Centrifuge the cells at 300 x g for 4 minutes and 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% CO ₂ . Gently replace medium after 24 hours with 5-10 mL of appropriate cell culture medium and continue culturing at 37°C with 5% CO ₂ . When appropriate (70-80% confluency), expand cell lines to a T75 flask using the subculturing procedures 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% CO ₂ .	
Recommended Cryopreservation Medium	45% RPMI 1640, 50% FBS, 5% DMSO	

Additional Information

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

