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HANDLING AND CULTURE

Culture medium

DMEM□w□4.5 □/□□□□□w□4 □□□ L-□□□□□w□3.7 □/□ NaHCO3□w□1.0 □□□□□□□□Cytion □□ 820300a□□

Incubation atmosphere	37°C, 5%CO2, □□□□□

Supplements

□□□□□□□ 10% FBS

Dissociation reagent	Accutase

Subculturing

□□□□□□□□□□□□□□□□□□□□ PBS □□□T25 □□□ 3-5 □□ PBS□T75 □□□ 5-10 □□□□□□ Accutase □□□□□□□T25 □□□ 1-2 □□□T75 □□□ 2.5 □□□□□□□□□□□□ 8-10 □□□□□□□□□□□□□ 10 □□□□□□□□□□□□□□□□□□□□ 300xg □□ 3 □□□□□□□□□□□□□□□□□□□□□□ □□□□□□□□□□□□□□□□□□□□

Fluid renewal	□□ 2 □ 3 □

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

Sterility

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Freeze medium	□□□□□□□□□□□□□□□ FBS□+10% DMSO □ CM-1□Cytion □□□□□□ 800100□□□□□□□□□ □□□□□□□□□□□□□□□□□CM-1□Cytion □□□□□□ 800100□□□□□□□□□□□□□□□□□□□□□□ □□□□□□□□□□□□□□□□□

Storage conditions

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Shipping conditions

□□□ -78 °C □□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□

DONOR

Donor age	72 □
Ethnicity	□□□□

Cytion GmbH
Nikola-Tesla-Str. 3
69124 Heidelberg
Tel: +49 (0)6221 405780
info@cytion.com
cytion.com

Registered office Heidelberg
VAT ID: DE283292917
Local court Mannheim - HRB 714394
CEO / Geschäftsführer: Jonathan Steubing

Banking information
Sparkasse Heidelberg
IBAN: DE35 6725 0020 0009 2076 86
BIC: SOLADES1HDB

LEGAL AND SAFETY

Safety precautions

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