A64-CLS □□

300199
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GENERAL INFORMATION

Description

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Tissue	□□□□
Disease	□□

Synonyms

- A-64CLS

CLASSIFICATION

Organism	Human
Sex	Male
Growth properties	Adherent
Biosafety level	1

CHARACTERISTICS

Tumourigenic	□□□□□□□□

IDENTIFIERS

Citation

A64-CLS□Cytion □□ 300199□

NCBI TaxID	9606
Cellosaurus	CVCL_5968

HANDLING AND CULTURE

Culture medium

EMEM□MEM Eagle□□□ 2 mM L-□□□□□□ 2.2 g/L $NaHCO_3$□□ EBSS□Cytion □□□□ 820100a□

Incubation atmosphere	37°C□5% CO_2□□□□□□

Supplements

□□□□□□□ 10% FBS □ 1% NEAA

Dissociation reagent	Accutase

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Subculturing
從貼壁細胞中移除舊培養基，並用不含鈣和鎂的PBS洗滌細胞。T25培養瓶使用3-5毫升PBS，T75培養瓶則使用5-10毫升。 接著，用 Accutase 完全覆蓋細胞，T25 培養瓶使用 1–2 毫升，T75 培養瓶則使用 2.5 毫升。將細胞於室溫下孵育 8–10 分鐘，使其脫附。 孵育後，將細胞與 10 毫升培養基輕柔混合以使其重新懸浮，然後以 300xg 離心 3 分鐘。棄去上清液，將細胞重新懸浮於新鮮培養基中，並轉移至已預先加入新鮮培養基的新培養瓶中。

Seeding density 1 × 10⁴ 個細胞/cm²

Fluid renewal 每 3 至 5 天

Post-thaw recovery 解凍後，將細胞以 5 × 10⁴ 個細胞/cm² 的密度接種於培養皿中，並讓細胞從冷凍過程中恢復，並附著至少 24 小時。

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
透過基於 PCR 的檢測及基於發光原理的支原體檢測方法，排除支原體污染的可能性。
為確保無細菌、真菌或酵母菌污染，每日均會對細胞培養物進行目視檢查。

Freeze medium 作為冷凍保存培養基，我們使用完全生長培養基（含 FBS）+ 10% DMSO 以確保解凍後的存活率，或使用 CM-1
（Cytion 型號 800100），該培養基含有經優化的滲透保護劑和代謝穩定劑，可提升細胞恢復率並減輕冷凍誘導的壓
力。

Storage conditions
若需長期保存，請將試管置於溫度約為 −150 至 −196 °C 的氣相液氮中。僅在轉移至液氮之前，可將試管暫時存放於 −80 °C 環境中，作為短期過渡步驟。

Shipping conditions
冷凍保存的細胞系會以乾冰冷藏，並裝於經驗證的隔熱包裝中，包裝內含有足夠的冷媒，以確保在運輸過程中溫度維持在約 −78 °C。收到貨物後，請立即檢查容器，並盡快將試管轉移至適當的儲存場所。

DONOR

Donor age 63年

Ethnicity 高加索人

LEGAL AND SAFETY

Safety precautions
若計劃將低溫試管存放在液態氮中以供日後解凍，必須嚴格遵守相關安全措施。 必須穿戴適當的防護手套和防護服，且在將冷凍樣本移入或移出液氮罐時，必須佩戴口罩或安全護目鏡。此舉旨在降低因取出時冷凍試管可能發生爆炸而導致受傷的風險，此類爆炸可能造成尖銳碎片飛濺。

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Warranty

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Material Transfer Agreement required	Yes