CHO/Human CD64 Stable Cell Line (High Expression) Data Sheet

➢ *Limited Use & License Disclosure*

BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE FOLLOWING TERMS OF LIMITED USE OF THIS CELL LINE PRODUCT.

- If the researcher is not willing to accept the terms of limited use of this cell line product, and the product is unused, ACRO will accept return of the unused product.
- Researchers may use this product for research use only, no commercial use is allowed. "Commercial use" means any and all uses of this product and derivatives by a party for profit or other consideration and may include but is not limited to use in: (1) product manufacture; and (2) to provide a service, information or data; and/or resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research.
- This cell line is neither intended for any animal or human therapeutic purposes nor for any direct human in vivo use. You have no right to share, modify, transfer, distribute, sell, sublicense, or otherwise make the cell line available for use to other researchers, laboratories, research institutions, hospitals, universities, or service organizations.
- ACROBIOSYSTEMS MAKES NO WARRANTIES OR REPRESENTATIONS OF ANY KIND, EITHER EXPRESSED OR IMPLIED, WITH RESPECT TO THE SUITABILITY OF THE CELL LINE FOR ANY PARTICULAR USE.
- ACROBIOSYSTEMS ACCEPTS NO LIABILITY IN CONNECTION WITH THE HANDLING OR USE OF THE CELL LINE.
- Modifications of the cell line, transfer to a third party, or commercial use of the cell line may require a separate license and additional fees. Please contact order.cn@acrobiosystems.com for further details.

CHO/Human CD64 Stable Cell Line (High Expression) Data Sheet

CHO/Human CD64 Stable Cell Line (High Expression)

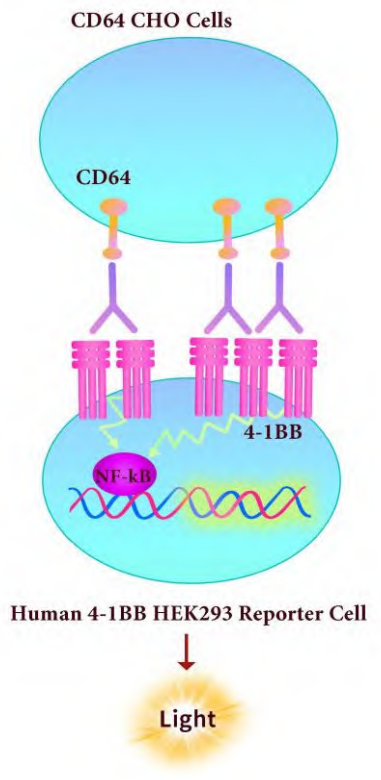
Catalog No.	Size
SCCHO-ATP062H	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The CHO/Human CD64 Stable Cell Line was engineered to express the receptor full length human CD64 (Uniprot: P12314-1), with different levels of CD64 expression (High, Medium, Low), which can be used to test agonist antibody whether in a CD64-dependent manner to strengthen the agonistic activity. When co-cultured with Human 4-1BB HEK293 Reporter Cell and anti-4-1BB agonist antibody, the anti-4-1BB antibody can be crosslinked, thereby strengthening 4-1BB pathway-activated luminescence.

• *Application*

- Useful for cell-based CD64 binding assay
- Useful for CD64-mediated crosslinking



CHO/Human CD64 Stable Cell Line (High Expression) Data Sheet

• *Cell Line Profile*

Cell line	CHO/Human CD64 Stable Cell Line (High Expression)
Host Cell	CHO
Property	Adherent
Complete Growth Medium	F-12K + 10% FBS
Selection Marker	Hygromycin B (20 μg/mL)
Incubation	37℃ with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- F-12K Nutrient Mixture (BasalMedia, Cat. No. L450KJ)

Note: If you are unable to obtain the specified F-12K Nutrient Mixture (BasalMedia, Cat. No. L450KJ) in China, you may use an alternative F-12K Nutrient Mixture (Gibco, Cat. No. 21127-022) or another suitable medium for culturing.

- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Hygromycin B (Invitrogen, Cat. No. 10687010)
- 0.25% Trypsin-EDTA (1X), Phenol Red (Gibco, Cat. No. 25200-056)
- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat. No. SH30256.01)
- Complete Growth Medium: F-12K + 10% FBS, 1%P/S
- Culture Medium: F-12K + 10% FBS, Hygromycin B (20 μg/mL), 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO_2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

CHO/Human CD64 Stable Cell Line (High Expression) Data Sheet

• *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium. Centrifuge at approximately 1000 rpm for 5 minutes.
4. Resuspend the cell pellet with 5 mL complete growth medium and transfer the cell suspension into a T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
5. Incubate at 37℃ with 5% CO_2 incubator until the cells are ready to be split.

• *Subculture*

1. Cell viability may be low after thawing, and full recovery may take up to a week. Monitor the cells daily until the culture reaches 80-90% confluency. At this point, remove and discard the spent medium. Avoid allowing the cells to become over-confluent to ensure optimal cell health.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 3 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 5-7 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of culture medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps.
5. Transfer appropriate aliquots of the cell suspension to a new T-75 flask. A subcultivation ratio of 1:6 to 1:10 is recommended. Adjust the ratio based on your specific culture system.
6. Incubate at 37℃ with 5% CO_2 incubator.
7. When the cell culture reaches 80-90% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

Note: After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition, transition to the culture medium containing the selection marker during subculturing.

CHO/Human CD64 Stable Cell Line (High Expression) Data Sheet

• *Cryopreservation*

1. When the cell culture reaches 80-90% confluency, remove and discard the spent medium.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 3 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 5-7 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of complete growth medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps. Count the viable cells.
5. Transfer the cell suspension to a centrifuge tube. Centrifuge at 1000 rpm for 5 min at room temperature to pellet the cells.
6. After centrifugation, discard the supernatant. Resuspend the cells in ice cold freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
7. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

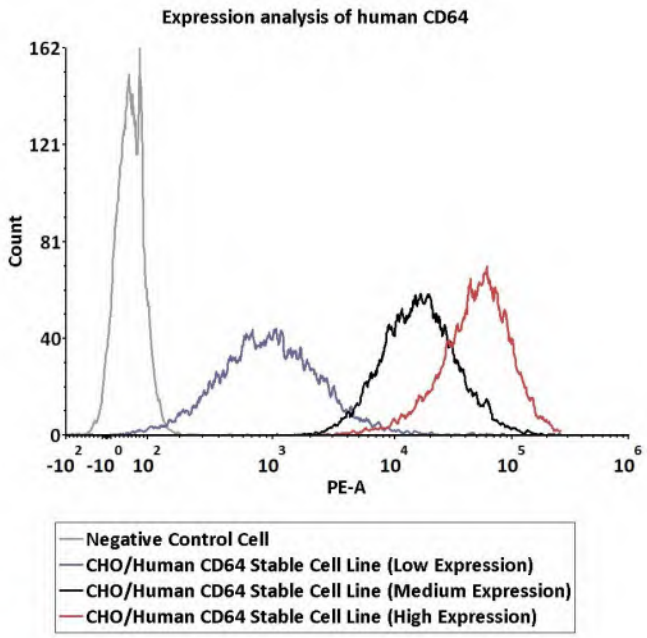
   Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *Storage Condition*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a –80°C freezer immediately upon receipt. If stored in a –80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

CHO/Human CD64 Stable Cell Line (High Expression) Data Sheet

- ***Receptor Assay***



Catalog No.	Stable Cell Line	MFI for CD64 (PE)
SCCHO-ATP062L	CHO/Human CD64 Stable Cell Line (Low Expression)	905.19
SCCHO-ATP062M	CHO/Human CD64 Stable Cell Line (Medium Expression)	15478.72
SCCHO-ATP062H	CHO/Human CD64 Stable Cell Line (High Expression)	49904.52

Fig1. Expression analysis of human CD64 on CHO/Human CD64 Stable Cell Line by FACS. Cell surface staining using PE-labeled anti-human CD64 antibody was performed on CHO/Human CD64 Stable Cell Line with different expression levels: CHO/Human CD64 Stable Cell Line (Low Expression); CHO/Human CD64 Stable Cell Line (Medium Expression); CHO/Human CD64 Stable Cell Line (High Expression).

CHO/Human CD64 Stable Cell Line (High Expression) Data Sheet

• *Related Products*

Products	Cat.No.
CHO/Human CD16a (158V) Stable Cell Line (Low Expression)	SCCHO-ATP059L
CHO/Human CD16a (158V) Stable Cell Line (Medium Expression)	SCCHO-ATP059M
CHO/Human CD16a (158V) Stable Cell Line (High Expression)	SCCHO-ATP059H
CHO/Human CD32b Stable Cell Line (Low Expression)	SCCHO-ATP060L
CHO/Human CD32b Stable Cell Line (Medium Expression)	SCCHO-ATP060M
CHO/Human CD32b Stable Cell Line (High Expression)	SCCHO-ATP060H
CHO/Human CD32a Stable Cell Line (Low Expression)	SCCHO-ATP061L
CHO/Human CD32a Stable Cell Line (Medium Expression)	SCCHO-ATP061M
CHO/Human CD32a Stable Cell Line (High Expression)	SCCHO-ATP061H
CHO/Human CD64 Stable Cell Line (Low Expression)	SCCHO-ATP062L
CHO/Human CD64 Stable Cell Line (Medium Expression)	SCCHO-ATP062M
CHO/Human PD-L1 Stable Cell Line (Low Expression)	SCCHO-ATP077L
CHO/Human PD-L1 Stable Cell Line (Medium Expression)	SCCHO-ATP077M
CHO/Human PD-L1 Stable Cell Line (High Expression)	SCCHO-ATP077H