

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

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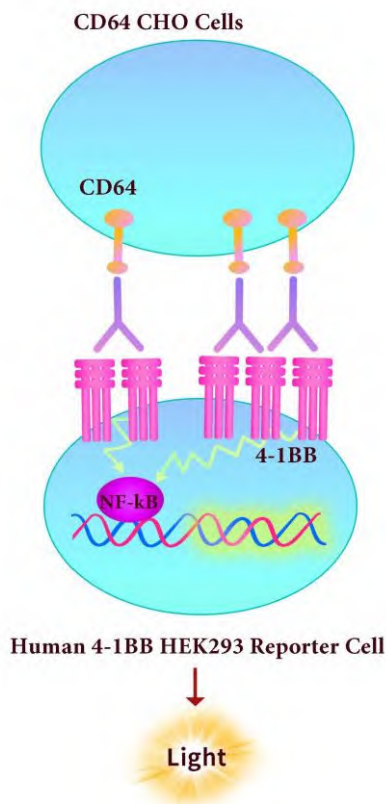
| Catalog No. | Size |
|---------------|------------------------------------------------|
| SCCHO-ATP062M | 2 × (1 vial contains ~5×10 ⁶ cells) |

• 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



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• Cell Line Profile

| | |
|------------------------|-----------------------------------------------------|
| Cell line | CHO/Human CD64 Stable Cell Line (Medium Expression) |
| Host Cell | CHO |
| Property | Adherent |
| Complete Growth Medium | F-12K + 10% FBS |
| Selection Marker | Hygromycin B (20 µg/mL) |
| Incubation | 37°C 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)
- CO₂ Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

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• *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°C with 5% CO₂ 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°C with 5% CO₂ 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.

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• *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.

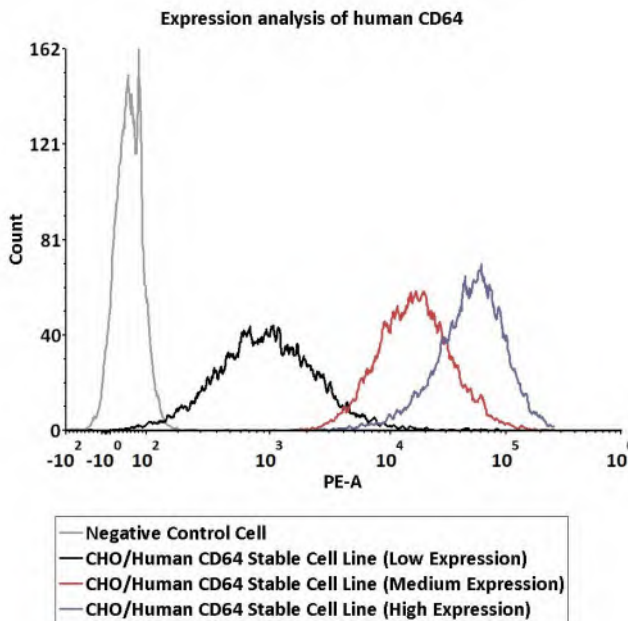
Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *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.

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• *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).

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• *Application*

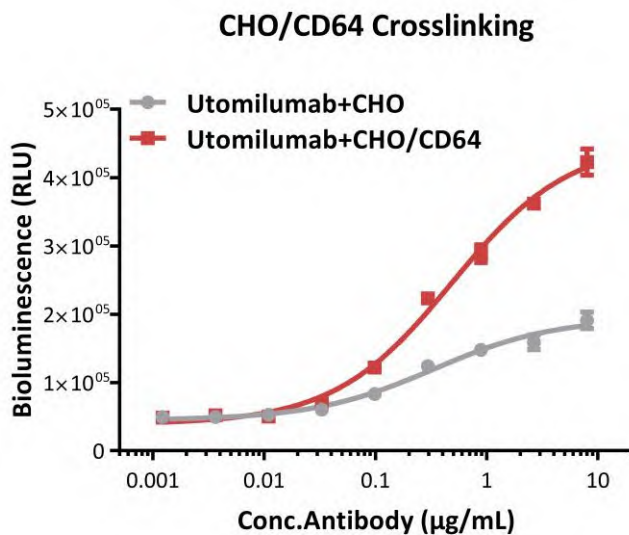


Fig2. Bioactivity analysis of anti-human 4-1BB antibody through CHO/Human CD64 Stable Cell Line (Medium Expression) crosslinking to test whether in a CD64-dependent manner to strengthen the agonistic activity. The EC50 of anti-human 4-1BB antibody is approximately 0.49 µg/mL through CHO/Human CD64 Stable Cell Line (Medium Expression) crosslinking.

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• *Related Products*

| <u>Products</u> | <u>Cat.No.</u> |
|-------------------------------------------------------------|-----------------------|
| 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 (High Expression) | SCCHO-ATP062H |
| 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 |