CHO/Human CD16a (158V) Stable Cell Line (Medium Expression)

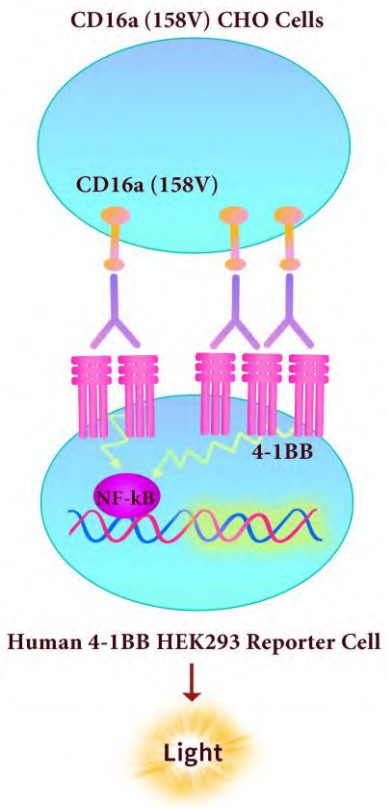
Catalog No.	Size
SCCHO-ATP059M	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The CHO/Human CD16a (158V) Stable Cell Line was engineered to express full length human CD16a receptor mutated to a Valine (V) at amino acid 158 with different levels of CD16a (158V) expression (High, Medium, Low), which can be used to test agonist antibody whether in a CD16a (158V)-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 CD16a (158V) binding assay
- Useful for CD16a (158V)-mediated crosslinking



CHO/Human CD16a (158V) Stable Cell Line (Medium Expression) Development Service Data Sheet

• *Cell Line Profile*

Cell line	CHO/Human CD16a (158V) Stable Cell Line (Medium Expression)
Host Cell	CHO
Property	Adherent
Complete Growth Medium	F-12K + 10% FBS
Selection Marker	Hygromycin (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 (Gibco, Cat.No.21127-022)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Hygromycin B (Invitrogen, Cat.No.10687010)
- Complete Growth Medium: F-12K + 10% FBS
- Culture Medium: F-12K + 10% FBS, Hygromycin (20 μg/mL)
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-Ⅱ)
- CO_2 Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

• *Recovery*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 4.0 mL complete growth medium and spin at approximately 1000 rpm for 5 minutes.
4. Resuspend cell pellet with 5 mL complete growth medium and transfer the cell suspension into 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. Remove and discard culture medium.
2. Wash the cells once with sterile PBS.
3. Add 3 mL of 0.25% trypsin to cell culture flask. Place the flask at 37°C for 5-7 minutes, until 90% of the cells have detached.
4. Add 6.0 to 8.0 mL of culture medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessel.
6. Incubate at 37℃ with 5% CO_2 incubator.

Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:10 is recommended.

Medium Renewal: Every 2 to 3 days.

• *Cryopreservation*

1. Remove and discard spent medium.
2. Detach cells from the cell culture flasks with 0.25% trypsin.
3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
4. Resuspend the cell pellets with complete growth medium and count viable cells.
5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
6. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transferring to liquid nitrogen storage.

• *Storage*

- **Product format:** Frozen
- **Storage conditions:** Liquid nitrogen immediately upon receipt

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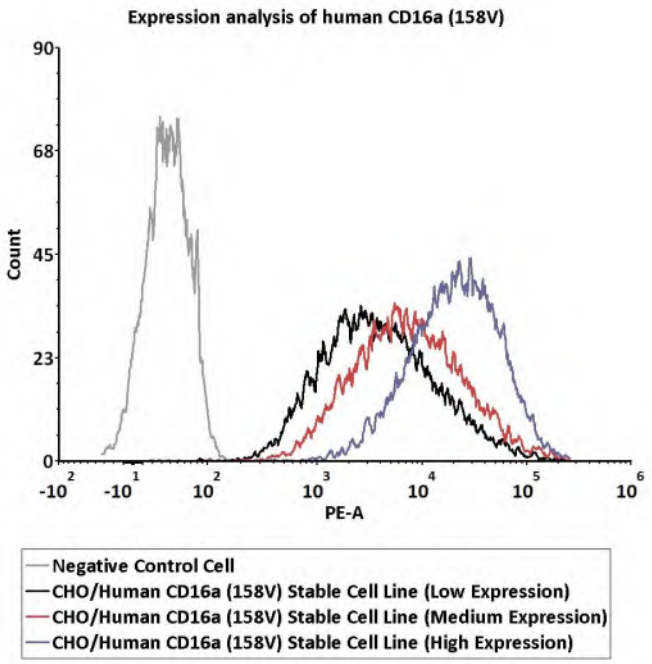
• *Receptor Assay*



Catalog No.	Stable Cell Line	MFI for CD16a (158V) (PE)
SCCHO-ATP059L	CHO/Human CD16a (158V) Stable Cell Line (Low Expression)	3430.17
SCCHO-ATP059M	CHO/Human CD16a (158V) Stable Cell Line (Medium Expression)	6751.79
SCCHO-ATP059H	CHO/Human CD16a (158V) Stable Cell Line (High Expression)	20546.84

Fig1. Expression analysis of human CD16a on CHO/Human CD16a (158V) Stable Cell Line by FACS. Cell surface staining using PE-labeled anti-human CD16a antibody was performed on CHO/Human CD16a (158V) Stable Cell Line with different expression levels: CHO/Human CD16a (158V) Stable Cell Line (Low Expression); CHO/Human CD16a (158V) Stable Cell Line (Medium Expression); CHO/Human CD16a (158V) Stable Cell Line (High Expression).

- ***Application***

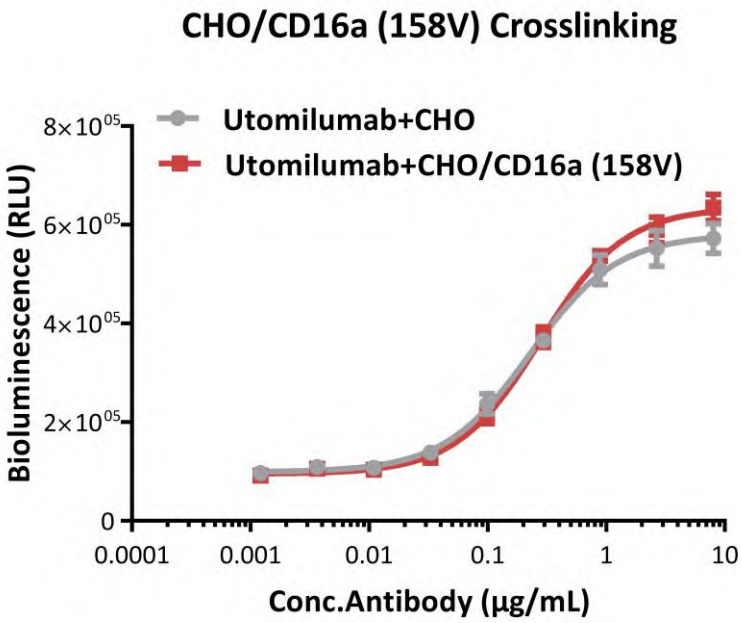


Fig2. Bioactivity analysis of anti-human 4-1BB antibody through CHO/Human CD16a (158V) Stable Cell Line (Medium Expression) crosslinking to test whether in a CD16a (158V)-dependent manner to strengthen the agonistic activity. The EC50 of anti-human 4-1BB antibody is approximately 0.23 μg/mL independent on CHO/Human CD16a (158V) Stable Cell Line (Medium Expression) crosslinking.

CHO/Human CD16a (158V) Stable Cell Line (Medium Expression) Development Service Data Sheet

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
CHO/Human CD16a (158V) Stable Cell Line (Low Expression) Development Service	SCCHO-ATP059L
CHO/Human CD16a (158V) Stable Cell Line (High Expression) Development Service	SCCHO-ATP059H
CHO/Human CD32b Stable Cell Line (Low Expression) Development Service	SCCHO-ATP060L
CHO/Human CD32b Stable Cell Line (Medium Expression) Development Service	SCCHO-ATP060M
CHO/Human CD32b Stable Cell Line (High Expression) Development Service	SCCHO-ATP060H
CHO/Human CD32a Stable Cell Line (Low Expression) Development Service	SCCHO-ATP061L
CHO/Human CD32a Stable Cell Line (Medium Expression) Development Service	SCCHO-ATP061M
CHO/Human CD32a Stable Cell Line (High Expression) Development Service	SCCHO-ATP061H
CHO/Human CD64 Stable Cell Line (Low Expression) Development Service	SCCHO-ATP062L
CHO/Human CD64 Stable Cell Line (Medium Expression) Development Service	SCCHO-ATP062M
CHO/Human CD64 Stable Cell Line (High Expression) Development Service	SCCHO-ATP062H
CHO/Human PD-L1 Stable Cell Line (Low Expression) Development Service	SCCHO-ATP077L
CHO/Human PD-L1 Stable Cell Line (Medium Expression) Development Service	SCCHO-ATP077M
CHO/Human PD-L1 Stable Cell Line (High Expression) Development Service	SCCHO-ATP077H