Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD32a-Medium Expression)

Catalog Number: CK-002

Components	Catalog No.	Size
Human 4-1BB (Luc) HEK293 Reporter Cell	CHEK-ATF073	2 × (1 vial contains ~5×10^6 cells)
CHO/Human CD32a Stable Cell Line (Medium Expression)	SCCHO-ATP061M	2 × (1 vial contains ~5×10^6 cells)

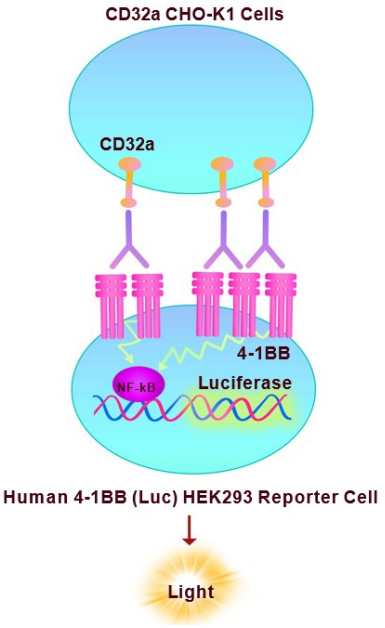
• *Description*

The Cell-based Kit consists of two engineered cell lines, Human 4-1BB (Luc) HEK293 Reporter Cell (Cat.No.CHEK-ATF073) and CHO/Human CD32a Stable Cell Line (Medium Expression) (Cat.No.SCCHO-ATP061M).

The Human 4-1BB (Luc) HEK293 Reporter Cell was engineered to not only express NF-κB signaling response element, but also express the receptor full length human 4-1BB（Gene ID: 3604), which can drive luciferase expressing systems by 4-1BB ligand/ agonist antibody stimulation. The CHO/Human CD32a Stable Cell Line was engineered to express full length human CD32a receptor (Gene ID: 2212), with different levels of CD32a expression (High, Medium, Low), which can be used to test agonist antibody whether in a CD32a-dependent manner to strengthen the agonistic activity. When co-cultured with Human 4-1BB (Luc) 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*

- Screen for Anti-human 4-1BB antibodies whether in a CD32a-dependent manner to strengthen the agonistic activity



Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD32a-Medium Expression) Data Sheet

• *Cell Line Profile of Human 4-1BB (Luc) HEK293 Reporter Cell*

Cell line	Human 4-1BB (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Hygromycin (20 μg/mL)
Incubation	37℃ with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Cell Line Profile of CHO/Human CD32a Stable Cell Line (Medium Expression)*

Cell line	CHO/Human CD32a 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

• *Cell Culture of Human 4-1BB (Luc) HEK293 Reporter Cell*

Materials Required

- DMEM medium (Gibco, Cat.No.11965-092)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Puromycin (InvivoGen, Cat.No.ant-pr-5b)
- Hygromycin B (Invitrogen, Cat.No.10687010)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Hygromycin (20 μg/mL), Puromycin (2 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 culture 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

• *Cell Culture of CHO/Human CD32a Stable Cell Line (Medium Expression)*

Materials Required

- 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°C 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 culture 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

- ***Receptor Assay***

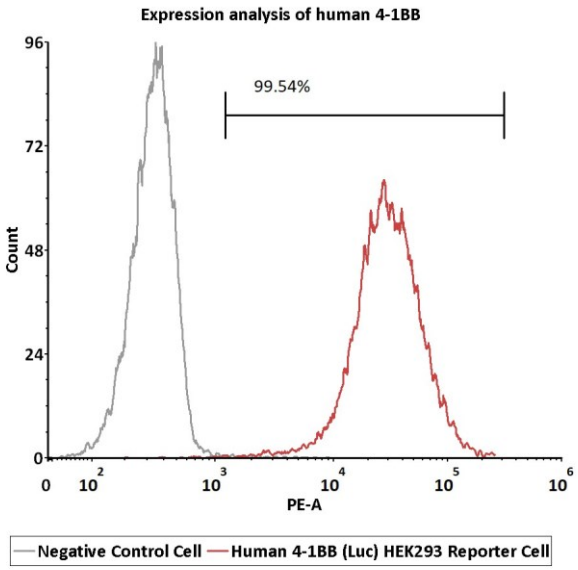
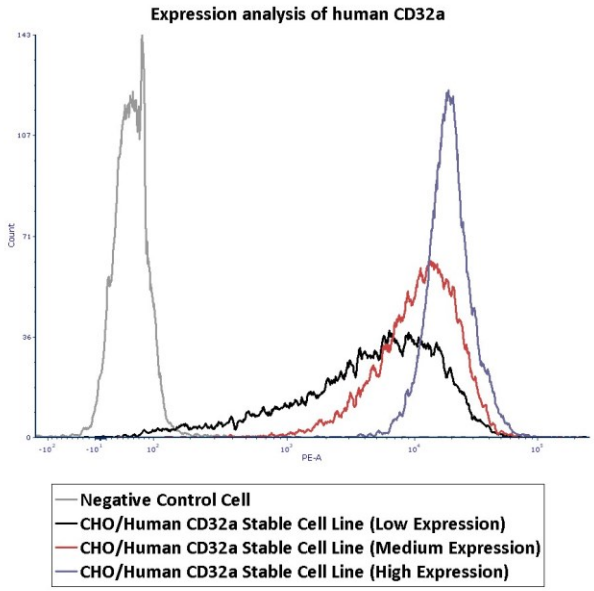


Fig1. Expression analysis of human 4-1BB on Human 4-1BB (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human 4-1BB (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human 4-1BB antibody.



Catalog No.	Stable Cell Line	MFI for CD32a (PE)
SCCHO-ATP061L	CHO/Human CD32a Stable Cell Line (Low Expression)	5196.88
SCCHO-ATP061M	CHO/Human CD32a Stable Cell Line (Medium Expression)	11336.11
SCCHO-ATP061H	CHO/Human CD32a Stable Cell Line (High Expression)	18309.90

Fig2. Expression analysis of human CD32a on CHO/Human CD32a Stable Cell Line by FACS. Cell surface staining using PE-labeled anti-human CD32a antibody was performed on CHO/Human CD32a Stable Cell Line with different expression levels: CHO/Human CD32a Stable Cell Line (Low Expression); CHO/Human CD32a Stable Cell Line (Medium Expression); CHO/Human CD32a Stable Cell Line (High Expression).

• *Application*

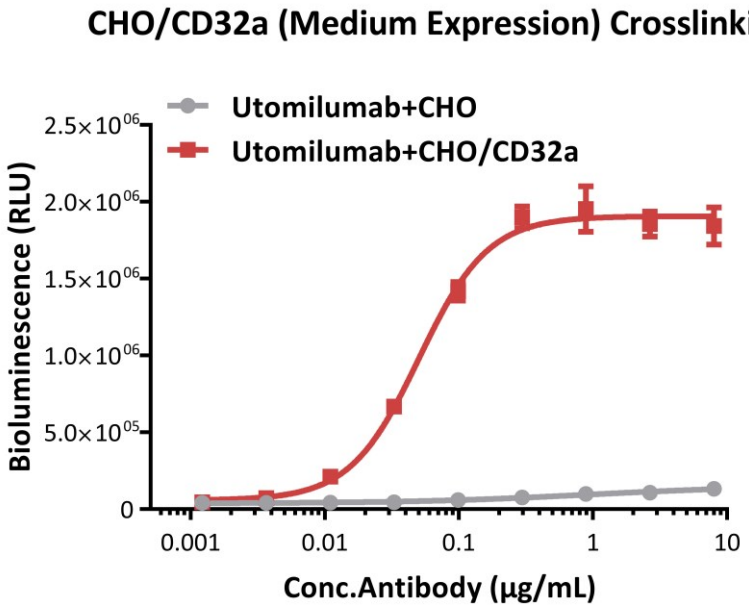


Fig3. Bioactivity analysis of anti-human 4-1BB antibody through CHO/Human CD32a Stable Cell Line (Medium Expression) crosslinking to test whether in a CD32a-dependent manner to strengthen the agonistic activity. The EC50 of anti-human 4-1BB antibody is approximately 0.05 μg/mL through CHO/Human CD32a Stable Cell Line (Medium Expression) crosslinking.

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD32a-Low Expression)	CK-001
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD32a-High Expression)	CK-003
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD16a (158V)-Low Expression)	CK-004
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD16a (158V)-Medium Expression)	CK-005
Cell-based Screening Kit for Anti-human 4-1BB Antibody (HumanCD16a (158V)-High Expression)	CK-006
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD32b-Low Expression)	CK-007
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD32b-Medium Expression)	CK-008
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD32b-High Expression)	CK-009
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD64-Low Expression)	CK-010
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD64-Medium Expression)	CK-011
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human CD64-High Expression)	CK-012
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human PD-L1-Low Expression)	CK-013
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human PD-L1-Medium Expression)	CK-014
Cell-based Screening Kit for Anti-human 4-1BB Antibody (Human PD-L1-High Expression)	CK-015