6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
**Tel:** 1.858.202.1401
**Fax:** 1.858.481.8694
**Email:** support@bpsbioscience.com

# Data Sheet
## CD27 CHO-K1 Stable Cell Line
## Catalog #60624

**Description**
CHO-K1 cell line expressing full length human CD27 (TNFRSF7). Expression is confirmed by real time qPCR, Western blot, and flow cytometry.

**Background**
CD27 is a member of the tumor necrosis factor (TNF) receptor superfamily, which includes the T cell co-stimulatory receptors OX40, 4-1BB and herpesvirus entry mediator (HVEM). CD27 is expressed on various types of T cells, B cells and a subset of natural killer cells. It activates NF-κB and MAPK/JNK signaling upon interaction with its TNF-like ligand, CD70, which is expressed by numerous tumor cells. Adaptor proteins TRAF2 and TRAF5 can also stimulate CD27 signaling. Activation of CD27 leads to lymphoid proliferation, differentiation, apoptosis, and the induction of long-term memory. The CD27/CD70 pathway is a key target for the development of treatments for cancer and inflammatory diseases.

**Application**
Screening for antibodies recognizing CD27 and screening antibodies for binding affinities. Since CHO-K1 cells do not express endogenous CD27, this cell line serves as an excellent model for studying changes in CD27-mediated signaling pathways, such as NF-κB, MAPK and SAPK/JNK.

**Host Cell**
Chinese Hamster Ovary Cells. Adherent epithelial cells.

**Format**
Each vial contains 2 x $10^6$ cells in 1 mL of 10% DMSO in FBS.

**Storage**
Store in liquid nitrogen immediately upon receipt.

**Culture Medium**
**Thaw Medium 3 (BPS Bioscience, #60186):** F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

**Growth Medium 3E (BPS Bioscience, #79553):** F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 100 µg/ml Hygromycin B

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**Recommended Culture Condition**

*Frozen Cells*: Prepare T-75 culture flask with 20 ml of pre-warmed Thaw Medium 3 **(no Hygromycin B)**. Quickly thaw cells in a 37°C water bath with constant and slow agitation. After cleaning the outside of the vial with 70% ethanol, immediately transfer the entire content to Thaw Medium 3 **(no Hygromycin B)**. Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% $CO_2$. 24-48 hours after incubation, change to fresh Thaw Medium 3 **(no Hygromycin B)**, without disturbing the attached cells. Continue to change medium every 2-3 days until reached desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture.

*Subculture*: When cells reach 90% confluency, remove the medium and wash twice with PBS (without Magnesium or Calcium). Treat cells with 2-3 mL of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml pre-warmed Growth Medium 3E **(contains Hygromycin B)** and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and resuspend cells in 10 ml of pre-warmed Growth Medium 3E **(contains Hygromycin B)**. Dispense 2 ml of the cell suspension into a new T75 flask containing pre-warmed 18 ml Growth Medium 3E **(contains Hygromycin B)**. Incubate cells in a humidified 37°C incubator with 5% $CO_2$. Freeze cells in freezing medium (10% DMSO in FBS) when cells reach 90% confluency. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks so cells are not used beyond passage 15.

**Mycoplasma Testing**

This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, Cat. #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, Cat. #LT07-518) was used as a positive control.

**Application References**

1. Akiba H *et.al.* (1998) CD27, a member of the tumor necrosis factor receptor superfamily, activates NF-κB and stress-activated protein kinase/ c-Jun N-terminal kinase via TRAF2, TRAF5, and NF-κB inducing kinase. *J. Biol. Chem.* **273:** 13353
2. Yamamoto H *et.al.* (1998) NF-κB activation in CD27 signaling: Involvement of TNF receptor-associated factor in its signaling and identification of functional region of CD27. *J. Immunology* **161:** 4753-4759

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**Quality Assurance**

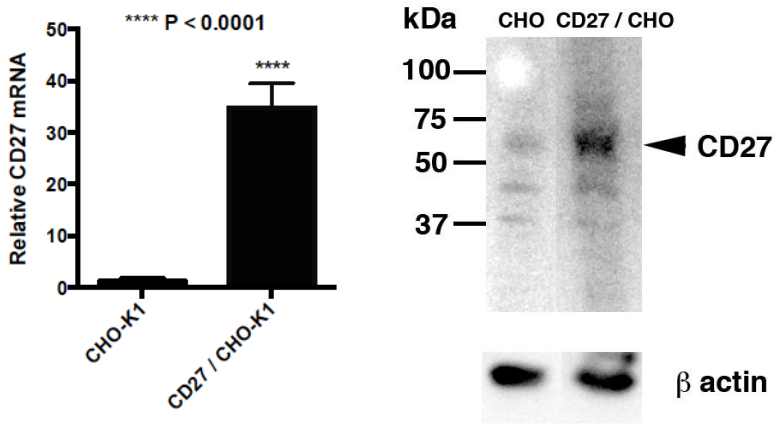


**Figure 1. Human CD27 expression in CHO-K1 cells**
(**Left**) Cells were seeded at semi-confluency in 24 well plates overnight and were treated by TRIZOL® (Thermo Fisher, Cat. #15596018) for RNA isolation, followed by reverse transcription and real-time PCR using the BioRad CFX96 Thermocycler. CD27 mRNA level was calculated with respect to β actin, using the standard $2^{-\Delta\Delta Ct}$ method. Error bar = S.D., n=4, ****P < 0.0001; Student's two-tailed unpaired t-test. (**Right**) Cells were seeded at semi-confluency in 6-well plates overnight before treatment with lysis buffer for protein extraction. CD27 protein expression was validated by Western blot using anti-human CD27 antibody (Santa Cruz Technology, # sc-25289). Anti-β-actin (Cell Signaling Technology, Cat. #3700) was used as a loading control.

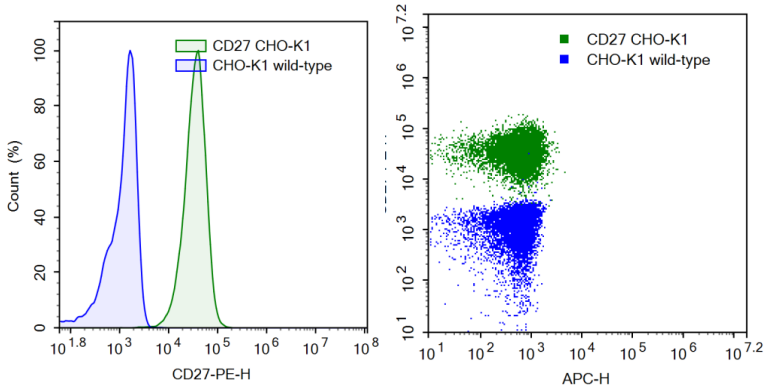


**Figure 2.**
CD27 surface expression in CHO-K1 cells was detected using a PE- conjugated anti-human CD27 antibody (clone MT271; Biolegend Cat. #356405). CHO-K1 wild-type was used as a negative control.

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**Vector and Sequence**
Human CD27 (NP_001233.1; Genbank Accession #BC012160) was cloned into the MCS of pIREShyg3 vector (Clontech, Cat. #631620).

MARPHPWWLCVLGTLVGLSATPAPKSCPERHYWAQGKLCCQMCEPGTFLVKDCDQHRKAA
QCDPCIPGVSFSPDHHTRPHCESCRHCNSGLLVRNCTITANAECACRNGWQCRDKECTECDP
LPNPSLTARSSQALSPHPQPTHLPYVSEMLEARTAGHMQTLADFRQLPARTLSTHWPPQRSL
CSSDFIRILVIFSGMFLVFTLAGALFLHQRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQE
DYRKPEPACSP

**Related Products**

| **Product** | **Cat. #** | **Size** |
|---|---|---|
| ONE-Step™ Luciferase Assay System | 60690-1 | 10 ml |
| ONE-Step™ Luciferase Assay System | 60690-2 | 100 ml |
| CD27, Fc fusion Protein | 71176 | 100 µg |
| CD70(CD27L), His-tag Protein | 71178 | 100 µg |

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