**Description**

The CD95 CHO cell line is a recombinant clonal stable CHO cell line constitutively expressing full-length human CD95 receptor (accession number: NM_000043.3). Surface expression of human CD95 was confirmed by flow cytometry. Each clonal cell line was selected for different levels of CD95 expression (High or Medium) to mimic varying CD95 expression levels in cancer cells.

**Background**

CD95 (Fas, APO-1, TNFRSF6) is a transmembrane cell-surface receptor with multifunctional regulatory effects in apoptosis and proliferation regulation. In some cellular contexts, interactions with the receptor ligand (CD95L) have been shown to activate JNK, ERK1, and NF-kB pathways to induce cell proliferation. In other cases, ligand interactions can trigger the release of a cytoplasmic death domain and induce apoptosis. This function is used by the immune system to kill cancer cells, and consequently, cancer cells can become resistant to CD95-induced apoptosis. The overexpression of CD95 in this cell line allows for the rapid testing of novel therapeutics that aim to increase CD95-mediated apoptosis in cancer cells.

**Application**

- Test compounds that activate the death domain of CD95 in cancer cells.
- Screen and validate antibodies against CD95 for drug discovery.
- Design CD95 binding assays to screen for CD95 ligands.

**Materials Provided**

| Components | Format |
|---|---|
| 2 vials of frozen cells | Each vial contains 2 x $10^6$ cells in 1 ml of cell freezing medium (BPS Bioscience #79796) |

**Parental Cell Line**

CHO-K1 cells, Chinese Hamster Ovary, epithelial-like cells, adherent

**Mycoplasma Testing**

The cell line has been screened to confirm the absence of Mycoplasma species.

**Materials Required but Not Supplied**

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

*Media Required for Cell Culture*

| Name | Ordering Information |
|---|---|
| Thaw Medium 3 | BPS Bioscience #60186 |
| Growth Medium 3B | BPS Bioscience #79529 |

*Materials Required for Cellular Assay*

| Name | Ordering Information |
|---|---|
| F-12K Medium (Kaighn's Modification of Ham's F-12 Medium) | ThermoFisher, #21127030 |
| ELANE (Elastase), Avi-His-Tag HiP™ Recombinant | BPS Bioscience #101141 |

| | |
|---|---|
| Recombinant Mouse Active Cathepsin C/DPPI Protein, CF | R&D Systems #2336-CY-010 |
| 96-well Flat Clear Bottom White Polystyrene TC-treated Microplates | Corning #3610 |
| Caspase-Glo® 3/7 Assay System | Promega #G8091 |

**Storage Conditions**

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage.
Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

**Media Formulations**

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37°C with 5% $CO_2$. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

*Media Required for Cell Culture*

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

*Growth Medium 3B (BPS Bioscience #79529):*
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 µg/ml of Hygromycin B.

*Media Required for Functional Cellular Assay*

*F-12K medium (ThermoFisher #*21127030*)*

**Cell Culture Protocol**

*Cell Thawing*

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 3 **(no Hygromycin)**.
   **Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.**
2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 3 **(no Hygromycin)**.
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% $CO_2$ incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 **(no Hygromycin)** and continue growing in a 5% $CO_2$ incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 3B **(contains Hygromycin)**.

*Cell Passage*

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3B and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3B **(contains Hygromycin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:15 twice per week.

*Cell Freezing*

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 3B and count the cells.
3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x $10^6$ cells/ml.
4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
5. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

**Validation Data**

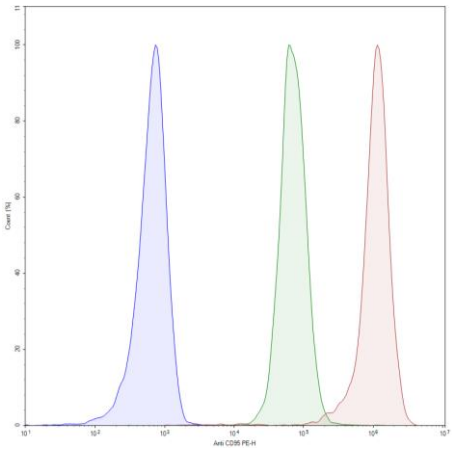

*Figure 1: Flow cytometry analysis of cell surface expression of human CD95 in CHO-K1 cells.*
High and medium expression CD95 CHO cells (red and green, respectively) or parental CHO cells (blue) were stained with PE-labeled Anti-CD95 Antibody (Biolegend #305608) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

**Functional characterization of CD95 CHO Cell Line**
The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.

*Assay Medium*:

Seeding cells:
Thaw Medium 3 (BPS Bioscience #60186)

Wash and Assay treatment:
F-12K Medium (Kaighn's Modification of Ham's F-12 Medium)

**A. ELANE – CD95 Functional Assay**

**Prior to assay:** Activate recombinant human ELANE (rhELANE):
Activation buffer: 50 mM MES + 50 mM NaCl, pH 5.5

**Protocol:**

1. Dilute rhELANE (BPS Bioscience #101141) to 50 µg/ml in Activation Buffer containing 50 µg/ml active rmCathepsin C.

2. Incubate for 2 hours at 37 °C to activate rhELANE.

3. Aliquot into multiple tubes to limit freeze/thaws and freeze at -80°C.

**Protocol:**

**Day 1:** Seed cells: seed 20,000 CD95 CHO cells in 100 µl of Thaw Medium 3 in each well of a clear-bottom white 96-well plate.
Incubate cells for 24 hours at 37°C and 5% $CO_2$.

**Day 2:** Wash cells: Carefully remove Thaw Medium 3 from the plate without disturbing the cells. Wash the plate once with 100 µl of F-12K Medium (no FBS). Remove the medium again and replace with 50 µl of F-12K basal medium (no FBS).

Treat Cells: Dilute the activated rhELANE in F-12K medium to the desired concentration, and add 50 µl of diluted rhELANE to the cells.

Incubate cells for 4 hours at 37°C and 5% $CO_2$.
*Note: Cell morphology will change at this point due to activation of the apoptosis pathway*.

Lyse cells using Promega Caspase-Glo® 3/7 Assay System according to the manufacturer's protocol.

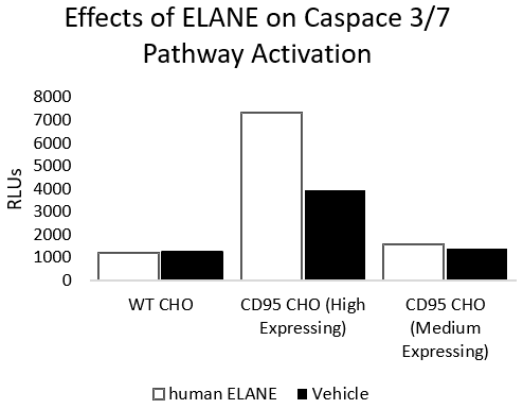


*Figure 2: Effects of ELANE on Caspace3/7 Pathway Activation.*
Effects of rhELANE on CD95 CHO cell line. CD95 CHO cells or parental CHO cells were treated with 2.5 µg/ml activated rhELANE or vehicle for 4 hours, then lysed using Caspase-Glo® 3/7 Assay System and read on a luminometer.

**Sequence**

CD95 sequence (accession number NM_000043.3)

MLGIWTLLPLVLTSVARLSSKSVNAQVTDINSKGLELRKTVTTVETQNLEGLHHDGQFCHKPCPPGERKARDCTVNGDEPDCVPC
QEGKEYTDKAHFSSKCRRCRLCDEGHGLEVEINCTRTQNTKCRCKPNFFCNSTVCEHCDPCTKCEHGIIKECTLTSNTKCKEEGSRS
NLGWLCLLLLPIPLIVWVKRKEVQKTCRKHRKENQGSHESPTLNPETVAINLSDVDLSKYITTIAGVMTLSQVKGFVRKNGVNEAKI
DEIKNDNVQDTAEQKVQLLRNWHQLHGKKEAYDTLIKDLKKANLCTLAEKIQTIILKDITSDSENSNFRNEIQSLV*

**References**

Cui, *et al.* (2021) *Cell* 184: 3163-3177
Peter, *et al*. (2015) *Cell Death & Differentiation* 22: 549-559
Strauss, *et al*. (2009) *J Exp Med*. 206(6): 1379–1393

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**Troubleshooting Guide**

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| CD95 CHO Datasheet (High Expression) | 78499-H | 2 Vials |
| ELANE (Elastase), Avi-His-Tag HiP™ Recombinant | 101141 | 25 µg |
| Cathepsin C, His-Tag Recombinant | 101393 | 10 µg |