NEUROMICS

GPCR Expressing CHO-K1

Catalog #: GPCR001

Cell #: >5x10^5 cells

Storage: Liquid Nitrogen until ready for culture.
While Culturing keep in 37°C CO_2 incubator

Product Format: Frozen Vial

GENERAL INFORMATION

CHO-K1 cells, derived from Chinese Hamster Ovary (CHO), are among the most extensively used mammalian cell lines in biotechnology and biomedical research. These cells are prepared to express G protein-coupled receptors (GPCRs) of interest, enabling them to serve as critical platforms for the production, study, and characterization of proteins. GPCRs are an expansive and essential family of integral membrane proteins that play a vital role in various signal transduction pathways and are consequently major targets for therapeutic drug development.

The exact expression parameters are set by the customer, with over 450 options available. To get the cells you need, please provide us with the protein accession number you are looking for. The protein accession number serves as a unique identifier assigned to a specific protein sequence within various biological databases, such as UniProt or GenBank. The lead time for the cells is 6-8 weeks, as they are prepared after ordering.

CHO-K1 cells exhibit a robust growth rate and are adaptable to various culture conditions, including suspension and adherent cultures, making them highly valuable for large-scale bioproduction processes.

Among the notable GPCRs often expressed in CHO-K1 cells are the β2-adrenergic receptor (β2-AR), the adenosine A2A receptor, various chemokine receptors (such as CXCR4), and opioid receptors. These receptors are frequently the focus of studies aimed at elucidating their roles in various disease states and as potential therapeutic targets.

At the end of the datasheet, there is more information on the advantages, applications, challenges, strategies, and assays related to the cells.

Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

HANDLING OF ARRIVING CELLS

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells into a -80°C freezer for short-term storage or liquid nitrogen tank for long-term storage.

3. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

4. When handling cell cultures, the application of stringent aseptic techniques is imperative. Maintaining a contamination-free environment is crucial for preserving the integrity and reliability of experimental results.

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This involves not only cleanliness but also the use of sterile tools, media, and working environments to prevent contamination from airborne particles, microbial agents, and other sources.

MEDIUM

We recommend GPCR CHO-K1 Growth Media (cat. GPCRM001) for these cells. The growth medium contains ingredients to support GPCR activity.

TIPS FOR SUCCESFUL CULTURING

- Monitor Cell Confluency: Regularly assess cell density to avoid over-confluency, as excessive confluence can stress cells and diminish protein production efficacy.

- Utilize Low-Passage Cells: For experimental purposes, select low-passage cells to ensure experimental consistency and reproducibility, mitigating variability due to cellular adaptation to culture conditions.

PROTOCOL FOR THAWING THE CELLS

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

1. To thaw, place the cryovial in a 37°C water bath, swirling gently until it thawed. Avoid overheating the cells.

2. Transfer the thawed cell suspension into a flask containing pre-warmed culture medium. This ensures the cells are in an appropriate environment for recovery and adaptation post-thaw.

3. Centrifuge the suspension at 200 × g for 5 minutes, effectively removing the cryopreservation medium that may be toxic to cells.

4. Gently resuspend the pellet in fresh culture medium and seed into a new flask for recovery and growth.

5. Place the flask back into the CO_2 incubator at 37°C and 5% CO_2, allowing the cells to recover and begin proliferating.

SUBCULTURING PROCEDURE

1. To prepare for passaging the cells, start by warming the culture medium to 37°C to maintain an ideal environment for the cells during handling.
2. Ensure that all procedures are conducted within a sterile biosafety cabinet to minimize contamination risks, maintaining aseptic techniques.
3. Remove the medium by carefully aspirating the spent culture medium from the flask, ensuring you do not disturb the cell layer to preserve cell integrity.
4. Wash the cells by adding 5-10 mL of PBS to the flask, swirling gently to dislodge any residual medium and cellular debris. Aspirate the PBS, ensuring the cells are clean and ready for detachment.
5. Introduce 2-3 mL of Cell Detachment Solution (cat. ADF001) into the flask. This solution effectively detaches the cells from the culture surface. Incubate the flask at 37°C for 2-5 minutes. Observe the cells under a microscope; they are ready for detachment when they become rounded and begin to lift from the surface. Once the cells are detached, immediately neutralize by adding an equal volume of neutralization solution to minimize any potential damage to the cells.

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6. Transfer the cell suspension into a sterile centrifuge tube for further processing. Centrifuge the tube at 200-300 × g for 5 minutes to pellet the cells at the bottom of the tube, allowing for easy resuspension of the cell pellet.
7. Carefully discard the supernatant. Resuspend the cell pellet in fresh culture medium to ensure a homogenous cell suspension. Count the cells using a hemocytometer or an automated cell counter to determine the appropriate seeding density for the next culture round.
8. Dilute the cell suspension to the desired density (e.g., 2-5 × 10^4 cells/cm^2) for optimal growth conditions.
9. Transfer the cell suspension into newly sterilized flasks or plates containing pre-warmed culture medium. Place the cultures in a CO_2 incubator at 37°C with 5% CO_2 to promote cell proliferation.

Medium Renewal: Change the culture medium every 2-3 days or sooner if it becomes yellow, indicating acidity. Fresh medium replenishes nutrients and growth factors necessary for cellular metabolism.

Passaging: Subculture when the cells reach 70-90% confluence, typically every 3-4 days. This prevents overgrowth and maintains healthy cell populations, ensuring consistent experimental results.

Monitoring: Regularly inspect cells under a microscope to assess morphology and check for evidence of contamination or abnormal growth patterns.

FREEZING PROCEDURE

1. Use the previously described method to obtain a healthy suspension of cells.
2. Resuspend the cell pellet in cryopreservation medium at a density of 1-5 × 10^6 cells/mL, ensuring conditions are favorable for long-term viability.
3. Distribute 1 mL of the cell suspension into sterile cryovials, clearly labeling each vial for easy identification and retrieval.
4. Utilize a controlled-rate freezer or a freezing container to slow-freeze the cells, minimizing ice crystal formation, which can compromise cell integrity.
5. Store the cryovials in liquid nitrogen, which provides ideal conditions for preserving the cell line for future research applications.

OTHER INFORMATION

CHO-K1 cells remain a pivotal tool in GPCR research, offering both advantages and challenges. Their role in advancing our understanding of GPCR biology and therapeutic potential cannot be overstated, and ongoing research continues to refine methodologies for their effective application.

ADVANTAGES OF CHO-K1 CELLS FOR GPCR EXPRESSION

- Mammalian-Like Post-Translational Modifications: CHO-K1 cells can perform complex post-translational modifications akin to those occurring in human cells, such as glycosylation, phosphorylation, and palmitoylation. These modifications are pivotal for the correct folding, stability, and functionality of GPCRs, ensuring that the receptors produced retain their biological activity essential for authentic signaling processes.
- High Protein Yield: One of the hallmark features of CHO-K1 cells is their ability to achieve high-level expression of proteins. This is particularly advantageous when large quantities of GPCRs are required for in-depth structural analysis, binding studies, or functional assays, thus facilitating a thorough understanding of receptor behavior.
- Scalability: These cells can be cultured in diverse bioreactor systems, allowing for substantial scalability. This attribute is crucial for industrial applications, including drug screening and the production of therapeutic proteins, where high cell densities and protein yields are necessary.
- Extensive Characterization: CHO-K1 cells have been well-studied, with a wide range of established protocols available for transfection, selection, culture, and protein expression. This well-documented background enables reproducibility and consistency in experimental design, fostering confidence in research outcomes.

APPLICATIONS OF GPCR EXPRESSING CHO-K1 CELLS

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- Drug Discovery: Since GPCRs are among the primary targets for new pharmaceuticals, CHO-K1 cells expressing specific GPCRs are instrumental in high-throughput screening (HTS) assays. These assays allow researchers to efficiently identify and characterize novel drug candidates that can modulate GPCR activity, which is critical for therapeutic development.
- Functional Studies: Scientists can use GPCR expressing CHO-K1 cells to explore intricate signaling pathways associated with GPCRs, including ligand-receptor interactions, receptor activation mechanisms, and downstream physiological effects. This research aids in the understanding of cell signaling and the physiological roles of various GPCRs in health and disease.
- Structural Biology: The GPCRs purified from GPCR expressing CHO-K1 cells are valuable for structural studies using techniques such as X-ray crystallography and cryo-electron microscopy (cryo-EM). These studies yield insights into the three-dimensional structures of GPCRs, enhancing our understanding of their mechanisms of action at a molecular level.
- Biophysical Studies: Techniques utilizing fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) are employed to investigate the dynamics and interactions of GPCRs in live cells. These studies deepen our understanding of GPCR behavior in a physiological context and are fundamental for developing targeted therapeutics.

CHALLENGES IN GPCR EXPRESSION

- Low Natural Expression Levels: GPCRs are often expressed at low levels within native tissues, presenting a challenge for achieving high levels of overexpression in CHO-K1 cells. This low expression can complicate functional assays and structural studies.
- Misfolding and Aggregation: The complexity of GPCR structure often leads to misfolding and aggregation, which can significantly hinder their functional integrity and overall yield. This challenge necessitates careful optimization during the expression and purification processes.
- Trafficking to the Cell Membrane: For GPCRs to fulfill their roles in signal transduction, they must properly localize to the cell membrane. Some receptors may require specific chaperones or additional post-translational modifications to ensure they are correctly trafficked and functional.

STRATEGIES TO IMPROVE GPCR EXPRESSION IN CHO-K1 CELLS

- Codon Optimization: Adjusting the codons used in the GPCR gene sequences can enhance transcription and translation efficiency within CHO-K1 cells, leading to improved protein production.
- Utilization of Tags: Incorporating affinity tags, such as FLAG, HA, or His-tags, can facilitate the detection, purification, and potentially the trafficking of GPCRs, streamlining the study of these proteins.
- Development of Stable Cell Lines: Establishing stable CHO-K1 cell lines that constitutively express GPCRs ensures consistent expression levels across various experiments, thereby improving reproducibility in research findings.
- Co-Expression of Chaperones: Co-expressing molecular chaperones alongside GPCRs can enhance proper folding and localization to the membrane, increasing the yield of functional receptors.
- Inclusion of Signal Peptides: Using signal peptides can promote more effective trafficking of GPCRs to the cell membrane, thereby maximizing their functional potential in signaling assays.

ASSAYS FOR GPCR FUNCTION IN CHO-K1 CELLS

- Cyclic AMP (cAMP) Assays: These assays determine the levels of cyclic AMP as a proxy for GPCR activity, providing crucial insights into the signaling pathways initiated by receptor activation.
- Calcium Mobilization Assays: By employing fluorescent dyes, these assays measure calcium release caused by GPCR activation, illuminating intracellular signaling cascades and cellular responses to receptor stimulation.
- Radioligand Binding Assays: This quantitative approach evaluates ligand-receptor interactions, enabling pharmacological characterization of GPCRs and facilitating drug discovery efforts.

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