Product description

CHO ICAM-1Fc cells secrete a dimeric human ICAM-1Fc protein. ICAM-1 (CD54) is a major ligand for leukocyte CD11/CD18 integrins and used in adhesion assays to evaluate integrin activity.

Name: CHO ICAM-1Fc Cell Line

Research Fields: Cell biology; Immunology

Organism: Hamster

Tissue: Ovary

Parental cell line: Chinese Hamster Ovary

Growth properties: Adherent

Model: Continuous, transgenic

Conditional: No

CRISPR edited: No

Production details: The chimeric wild-type ICAM-1Fc protein consists of the five extracellular domains of ICAM-1 joined to the hinge, CH2 and CH3 domains of IgG1. CHO cells were transfected using DEAE-dextran and the secreted proteins were collected in serum-free medium for 4 days. The proteins were purified on a Protein A Sepharose affinity column using standard methods.

Cellosaurus ID: CVCL_AS85

Contributor(s)

Inventor: Nancy Hogg
Institute: Cancer Research UK, London Research Institute: Lincoln's Inn Fields

Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Recommended medium: Glasgow MEM supplemented with 1% (v/v) MEM non-essential amino acids, 1% (v/v) L-glutamate and L-asparagine, 1 mM sodium pyruvate, 1× nucleosides, 100 IU/mL penicillin, 100 µg/mL streptomycin, and optionally 5% (v/v) dialysed foetal bovine serum

Additional supplements: 0.1 mM methionine sulfoximine, and 5 mM sodium butyrate.

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

Handling instructions

1. Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath when the **ice crystals are slightly dissolved** and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Add the cell suspension to 20 mL of prewarmed culture medium T250 flask.
5. When confluent, trypsinize and split into two T750 flasks.
6. On the day after splitting the cells, supplement with 0.1 mM methionine sulfoximine.
7. Continue splitting the cells 1:6 at confluence. When cells have reached a sufficient number (approximately 30 flasks), wash into culture medium with 0.1 mM methionine sulfoximine and 5 mM sodium butyrate, but without serum.
8. After approximately 10 days in serum-free culture, or when all the cells have died, harvest the medium, spin at 2000 rpm for 10 minutes to remove cell debris and filter through a 0.2 uM filter unit.
9. Isolate the ICAM-1Fc protein with a Sepharose A affinity column.
10. Wash the column with PBS. Elute 2 ml fractions with 0.1M citrate buffer pH3 into 600 ul 1M Tris pH8.5.
11. Concentrate the fractions containing protein using a Centricon 30 and wash into PBS.

References

- Stanley et al. 2008. EMBO J. 27(1):62-75. PMID: 18079697
- Smith et al. 2005. J Cell Biol. 170(1):141-51. PMID: 15983060
- Smith et al. 2003. J Cell Sci. 116(Pt 15):3123-33. PMID: 12799414

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: CHO ICAM-1Fc Cell Line, was invented by Nancy Hogg at Cancer Research UK, London Research Institute: Lincoln's Inn Fields (CancerTools #152756).