

BioWhittaker® UltraCHO™ Serum-free Cell Medium

Instructions for Use

Introduction

UltraCHO™ Medium was developed as a medium to support the growth of Chinese hamster ovary (CHO) cells and their recombinant derivatives. These cells are widely used in the production of recombinant proteins for use in therapeutic and diagnostic applications.

UltraCHO™ Medium is composed of a modified DMEM:F-12 base which is optimized to support the growth of transfected and non-transfected CHO cells. The formulation is supplemented with insulin, transferrin and proprietary purified proteins. This product is available as a complete 1X liquid (cat. no. 12-724) or powder (cat. no. 15-724) with a frozen supplement (cat. no. 17-811). UltraCHO™ Medium contains less than 300 µg/ml protein. UltraCHO™ Medium is manufactured under FDA cGMPs.

Partial List of Cell Types Cultivated with UltraCHO™ Medium:

Cell type

Transfected and non-transfected CHO cell lines
HeLa cells (suspension or attached)
Human leukemia cell lines

Instructions for Use

- UltraCHO™ Medium powder base, cat. no. 15-724
- UltraCHO™ Medium 100X supplement, cat. no. 17-811
- UltraCHO™ Medium base is a protein-free powdered medium designed to support the growth of CHO cells in either monolayer or suspension culture. A 100X frozen protein supplement (cat. no. 17-811) is recommended for use in conjunction with the UltraCHO™ Medium base formulation. The frozen supplement is non-sterile and should be added to the reconstituted UltraCHO™ Medium base prior to sterilization by filtration.

UltraCHO™ Medium base contains L-glutamine and is osmotically correct for the cultivation of

mammalian cells. The base may be used without the addition of the protein supplement or with a reduced amount of supplement. The success of this reduction in the protein supplement is dependent on the nutritional requirements of the user's transfected CHO line.

Please note: UltraCHO™ Medium liquid (cat. no. 12-724) is a 1X ready-to-use product which requires no further protein supplementation.

I. Preparation of UltraCHO™ Medium from Powder and 100X Supplement

1. Powdered media mixtures are extremely hygroscopic and must be protected from atmospheric moisture. The entire contents of each package should be used immediately after opening. Preparing medium in concentrated form is not recommended. Some free-base amino acids have low solubility coefficients and insoluble salt complexes may precipitate in concentrated solution.
2. Measure out 90% of the final required volume of water for cell culture (cat. no. 17-724). For example, 900 ml for final volume of 1000 ml. Select an appropriate container as close in size to the final volume as possible. Water temperature should be 15-20°C.
3. While gently stirring the water, add the UltraCHO™ Medium base powder and rinse the empty package with a small amount of water for cell culture.
4. Allow the UltraCHO™ 100X Supplement to thaw completely. Mix well and add 10 ml of the supplement per liter of the UltraCHO™ Medium base mixture.
5. Allow the mixture to stir for 20-30 min. **The pH OF UltraCHO™ Medium is self-adjusting. The addition of sodium bicarbonate is not required. Please do not attempt to adjust pH of the product.**
6. Add additional water for cell culture to bring the medium to final volume.

7. Sterilize immediately by filtration, using a membrane with a porosity of 0.22 microns or less. To reduce CO₂ loss, positive pressure (3-15 psi) should be used for filtration. An inert gas, such as nitrogen, should be used as a pressure source. CO₂ is not recommended as it will alter the pH of the medium.
8. Aseptically dispense the UltraCHO™ Medium into sterile containers. Store the liquid refrigerated at 2-8°C. Protect the liquid from long term exposure to intense light.

II. Growth of Cells in UltraCHO™ Medium

1. Adaptation of CHO cells to UltraCHO™ Medium may not be necessary. If adaptation is required, we recommend an initial split ratio of 1:2 into UltraCHO™ Medium followed by sequential splits of 1:5 until the culture is adapted to the new medium. During the weaning process, the cell concentration should be maintained above 3.0 x 10⁵ per ml.
2. Wash the monolayer of CHO cells with Trypsin/Versene® (cat. no. 17-161) or HBSS without calcium or magnesium (cat. no. 10-543) and immediately decant.
3. Add Trypsin/Versene® to the monolayer (5 ml/150 cm² flask) and incubate at room temperature for approximately one minute or until the cells have detached from the flask.
4. UltraCHO™ Medium contains a trypsin inhibitor, however, if the cells are not to be diluted more than 1:10 in fresh UltraCHO™ Medium, the residual trypsin should be removed by centrifugation at approximately 350 x g for 5 - 10 minutes.
5. After centrifugation, pour off the supernatant and resuspend the cell pellet in an appropriate volume of UltraCHO™ Medium. Triturate the cells to ensure a monodispersed cell suspension.
6. Perform a cell count and adjust the cell density. The suggested plant density for the cells is 5.0 x 10⁴ cells/cm² growth area.

Miscellaneous

1. Doubling time of CHO-K1 cells in UltraCHO™ Medium is approximately 17.5 hours.
2. This formulation should be stored at 2-8°C in the dark.
3. CHO cells frozen in serum-containing medium can be reconstituted directly into UltraCHO™ Medium.

4. CHO cells may also be cryopreserved in UltraCHO™ Medium plus 7.5% v/v DMSO.

Storage

Liquid media: 2°C to 8°C, dark
 Powdered medium: 2°C to 8°C
 Supplement: -10°C to -20°C

Product Use Statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering Information

Catalog number	Description	Size
12-724Q	UltraCHO™ Medium; serum-free CHO cell medium 1X liquid with L-glutamine	1 L
12-751F	UltraCHO™ Medium; serum-free CHO cell medium 1X liquid without nucleosides	500 ml
15-724D	UltraCHO™ Medium; serum-free CHO Cell medium powdered medium	1 x 10 L
15-724F	UltraCHO™ Medium; serum-free CHO Cell medium powdered medium	1 x 50 L
17-811A	UltraCHO™ Supplement non-sterile	100 ml