

CHOgro® Expression Medium, Dry Powder (10 L)

Quick Reference Protocol

Instructions for MIR 6201

SDS and Certificate of Analysis available at mirusbio.com/6201



SPECIFICATIONS

| | |
|------------------------------|--|
| Storage | Store CHOgro® Medium Dry Powder at 4°C, protected from light and moisture. |
| Product Guarantee | As indicated on product label, when properly stored and handled. |
| Product Configuration | Prepares 10 L of CHOgro® Expression Medium. |

► HYDRATION OF CHOgro® EXPRESSION MEDIUM, DRY POWDER

CHOgro® Expression Medium is a chemically defined, serum-free growth medium that permits high density growth and large-scale transfection of suspension CHO cells. Many suspension CHO cells (e.g. FreeStyle™ CHO-S®) readily adapt to CHOgro® Expression Medium, thus eliminating the time and labor typically required for a sequential adaptation process.

The following protocol describes how to prepare 1000 ml of liquid medium from CHOgro® Expression Medium, Dry Powder. Adjust quantities accordingly for different final volumes.

A. Hydration of CHOgro® Expression Medium, Dry Powder

1. While stirring, add 19.25 g of CHOgro® Medium Dry Powder to 900 ml cell culture grade water at a temperature between 22-25°C. NOTE: Room temperature or colder water can be used but will increase the solubilization time.
2. Stir the solution for 20 minutes or until the powder is fully dissolved.
3. Add 3.2 grams of sodium bicarbonate and mix until fully dissolved.
4. Bring the final solution volume to 1000 ml with cell culture grade water. Mix the solution for an additional 15-30 minutes.

B. Verify pH and filter hydrated CHOgro® Expression Medium

1. Verify that the pH of the final solution is between 7.0 and 7.2. If necessary, carefully adjust the pH with 1 N NaOH or 1 N HCl.
2. Verify that the osmolarity of the solution is between 260-300 mOsm/kg.
3. Filter sterilize CHOgro® Expression Medium with a 0.2-micron filter into the desired container.

C. Final media preparation

1. Prior to use, hydrated CHOgro® Expression Medium requires the following supplementation:

| Media Supplements | Per 1000 ml |
|---|-------------|
| L-Glutamine (200 mM stock solution, MIR 6240) | 20 ml |
| Poloxamer 188 Solution (10% stock solution, MIR 6230) | 30 ml |

2. Store hydrated, supplemented media at 4°C, protected from light.

► CHOgro® High Yield Expression System for High Titer Protein Production

The CHOgro® High Yield Expression System (MIR 6270) is an optimized platform for transient, high titer protein production in suspension CHO-derived cells. This system consists of CHOgro® Expression Medium, L-Glutamine and Poloxamer 188 medium supplements, *TransIT-PRO*® Transfection Reagent, CHOgro® Titer Enhancer and CHOgro® Complex Formation Solution. With the CHOgro® High Yield Expression System, high titers of therapeutic candidates for preclinical studies can be achieved. For more information, go to www.mirusbio.com/chogro.



Adaptation of suspension CHO cells to CHOgro® Expression Medium

From Cryopreserved Cell Stock

When bringing suspension CHO cells out of cryopreservation, use supplemented CHOgro® Expression Medium to dilute cells immediately post-thaw to a density of 1×10^6 cells/ml. Incubate cells in a shake flask at an appropriate rpm (e.g. 125 rpm for a 1.9-cm orbital throw) at 37°C in 8% CO₂. Monitor cell growth and viability daily. When viability reaches > 95% and the cells are doubling every ≤ 24 hours, the cells are fully adapted.

From Ongoing Culture

If cells are being cultured in an alternate media formulation, centrifuge the cells at $300 \times g$ for 5 minutes and resuspend the cell pellet in 100% CHOgro® Expression Medium at a density of 2×10^6 cells/ml. Incubate cells in a shake flask at an appropriate rpm (e.g. 125 rpm for a 1.9-cm orbital throw) at 37°C in 8% CO₂. Monitor cell growth and viability daily. When viability reaches > 95% and the cells are doubling every ≤ 24 hours, the cells are fully adapted.

NOTE: For CHO-S® cells grown in FreeStyle™ CHO Expression Medium, a 24 hour adaptation period to CHOgro® Expression Medium is sufficient for high titer transient protein production.

Maintenance of Suspension CHO cells in CHOgro® Expression Medium

For best results, subculture CHO suspension cells to a density of $1 - 3 \times 10^6$ cells/ml. DO NOT allow cells to grow to a density higher than 1×10^7 cells/ml or lower than 2.5×10^5 cells/ml during continuous culture. Subculture every 1-4 days to maintain desired cell density.

For Research Use Only

CHOgro® Expression Medium Dry Powder is animal-origin-free.