ClonaCell™-CHO CD Medium

Chemically defined, animal component-free, serum-free, protein-free, glutamine-free semi-solid methylcellulose-based medium for selecting and cloning CHO cells

Catalog #03815 90 mL

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Product Description

ClonaCell™-CHO CD Medium is a methylcellulose-based semi-solid medium recommended for selection and cloning of Chinese hamster ovary (CHO) cells. This medium is chemically defined, protein-free, and animal component-free. It does not contain L-glutamine, selection agents, or phenol red and is compatible with dihydrofolate reductase (DHFR) and glutamine synthetase (GS) selection systems.

Benefits of semi-solid cloning:

- Individual clones and their progeny remain localized together in a semi-solid matrix as they grow to form distinct colonies. This prevents the loss of rare, high-producing clones by overgrowth from faster-growing cells, as can occur during selection in a liquid medium, and facilitates the isolation of a diverse set of clones with a wide range of growth rates and productivities to be obtained for downstream screening.
- Colonies obtained from semi-solid medium have a high probability of monoclonality, allowing clonal cell lines to be generated in less time and using fewer resources than with limiting dilution cloning.
- Colonies can be easily picked from the semi-solid medium by manual or robotic methods and dispersed into a liquid growth medium for screening and expansion.

Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
ClonaCell™-CHO CD Medium	03815	90 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

Protect product from light. ClonaCell™-CHO CD Medium does not contain antibiotics.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
ClonaCell™-CHO CD Liquid Medium	03817
Blunt-End Needles, 16 Gauge	28110
12 mL Syringe Luer Lock Tip	Covidien 8881512878
100 mm Petri Dishes (non-tissue culture-treated)	27110

Directions for Use

Please read the entire protocol before proceeding.

CHO cells should be cultured in a chemically defined, protein-free liquid medium (e.g. ClonaCell™-CHO CD Liquid Medium) before performing selection and cloning in ClonaCell™-CHO CD Medium.

The selection and cloning protocol can be divided into four sequential sections:

I. Preparation of supplemented semi-solid medium (addition of selection agents and supplements to ClonaCell™-CHO CD Medium)

II. Suspending cells in supplemented semi-solid medium (addition of CHO cells to semi-solid medium)

III. Plating and culturing cells suspended in semi-solid medium

IV. Picking and expansion of individual colonies

I. Preparation of Supplemented Semi-Solid Medium

1. The day before use, thaw ClonaCell™-CHO CD Medium at 2 - 8°C overnight. Do not thaw in a 37°C water bath.
2. On the day of use, warm the fully thawed medium in a 37°C incubator for at least 1 hour. Do not warm in a 37°C water bath.
3. Determine the volume of ClonaCell™-CHO CD Medium required for the experiment based on the number of dishes to be used (Table 1) or the number of cells to be plated (Table 2).

   NOTE: If the entire ClonaCell™-CHO CD Medium bottle is to be used in a single experiment, additives (selection agents and supplements) and cells can be added directly to the bottle.

   For smaller volumes, aliquot the contents of the bottle either before or after addition of selection agents and supplements, but before addition of cells. This may be achieved by using a blunt-end 16-gauge needle attached to a 12 mL disposable syringe to dispense appropriate volumes into 50 mL tubes. For example, when 5 mL of additives have been added to an entire 90 mL bottle of ClonaCell™-CHO CD Medium, aliquot 9.5 mL of the supplemented semi-solid medium into each 50 mL tube. If additives have not been added to the bottle, aliquot 9 mL into each tube. Aliquots that are not used on the same day can be stored at -20°C for later use.

Table 1. Preparation of Supplemented Semi-Solid Medium

VOLUME OF ClonaCell™-CHO CD MEDIUM	VOLUME OF ADDITIVE SOLUTION (selection agents and supplements)*	VOLUME OF CELLS*	TOTAL VOLUME OF SEMI-SOLID CELL SUSPENSION	NUMBER OF 100 mm DISHES TO BE PLATED
90 mL (1 bottle)	5 mL	5 mL	100 mL	10
45 mL	2.5 mL	2.5 mL	50 mL	5
18 mL	1 mL	1 mL	20 mL	2
9 mL	0.5 mL	0.5 mL	10 mL	1

*The 1:9 (v/v) ratio of additives and cells:ClonaCell™-CHO CD Medium gives an appropriate viscosity for optimal cell growth and morphology.

4. Prepare the additive solution by adding the desired selection agents (e.g. antibiotics) and supplements (e.g. L-Glutamine; Catalog #07100) to a protein-free liquid medium suitable for culturing CHO cells (e.g. ClonaCell™-CHO CD Liquid Medium). See Table 1 for the required volume of additive solution.

   NOTE: The optimal concentration of selection agents should be determined experimentally by titration or based on previous experience with cloning cells in liquid medium. The addition of L-glutamine is essential for growth of CHO cells, except when the GS selection system is to be used.
5. Add the additive solution to ClonaCell™-CHO CD Medium (See Table 1 for required volumes). Replace the cap and shake vigorously for 1 - 2 minutes. The medium should appear opaque. Let the medium sit at room temperature (15 - 25°C) for 15 minutes to allow bubbles to rise to the surface.

II. Suspending Cells in Supplemented Semi-Solid Medium

The cells to be plated can be transferred directly from a culture flask, or harvested and resuspended in an appropriate medium according to standard protocols. For subcloning experiments, the viability of the cells should be greater than 90% and they should be in a logarithmic growth phase. It is important that the cells are in a single-cell suspension at the time of plating. If cells are clumped together, individual colonies may originate from more than one cell and the probability of monoclonality will be reduced.

1. Count viable CHO cells using a hemocytometer combined with Trypan Blue (Catalog #07050) staining, or an automated cell counter.

   NOTE: It is important to count cells prior to plating in ClonaCell™-CHO CD Medium. An inaccurate cell count may result in a suboptimal plating density.
2. Dilute the cells in a liquid medium suitable for culturing CHO cells (e.g. ClonaCell™-CHO CD Liquid Medium) to obtain the liquid cell suspension. See Table 1 for required volumes.

   NOTE: Prepare the cells at a range of cell densities to ensure that a suitable number of colonies is obtained per dish. The appropriate cell density range needs to be determined experimentally by the user, as it will vary depending on factors such as the transfection efficiency, selection method, and the condition of the cells at the time of plating. Refer to Table 2 for recommended cell plating densities.

Table 2. Recommended Plating Densities for CHO Cells

PROCEDURE	DESIRED NUMBER OF COLONIES PER 100 mm DISH	RANGE OF VIABLE CELLS PER 100 mm DISH*	EXAMPLE OF CELL DENSITIES PER 100 mm DISH
Selection and cloning after transfection	50 - 200	25,000 - 2,500,000	25,000 250,000 1,000,000 2,500,000
Recloning established cell lines	50 - 200	2000 - 20,000	2000 5000 10,000 20,000

*Plating at several cell densities is recommended as plating efficiencies may vary between cell lines and culture conditions.

3. Add the liquid cell suspension to the previously prepared and supplemented semi-solid medium (section I).
4. Replace the cap and shake the semi-solid cell suspension vigorously for 1 - 2 minutes. The medium should appear opaque. Let the medium sit at room temperature (15 - 25°C) for 15 minutes to allow bubbles to rise to the surface.

III. Plating and Culturing Cells Suspended in Semi-Solid Medium

1. Using a blunt-end 16-gauge needle attached to a 12 mL disposable syringe, plate 10 mL of the cell suspension (prepared in section II) per 100 mm dish. Tilt the dish gently to distribute the medium evenly, so that it covers the entire bottom of the dish.

   NOTE: Avoid introducing bubbles when expelling the semi-solid cell suspension from the syringe.
2. Repeat step 1 for each dish to be plated.
3. Incubate the dishes (with lid on) for 10 - 14 days at 37°C in 5% CO_2 and ≥ 95% humidity. Do not disturb the dishes. Early removal or disturbance of the dish, even briefly, may result in the formation of diffuse colonies.

   NOTE: To maintain appropriate hydration of the cultures, place the dishes inside a larger covered culture dish (e.g. 245 mm x 245 mm Square Treated Tissue Culture Dish; Catalog #27140) along with an additional uncovered 100 mm dish containing sterile water.

IV. Picking and Expansion of Individual Colonies

1. Examine the dishes for the presence of colonies that are visible to the naked eye.

   NOTE: A longer incubation time may be needed for some cell lines or selection systems; re-examine dishes after an additional 7 days if needed.
2. Pick colonies (colony diameter typically 0.5 - 1.0 mm) using a 200 µL sterile pipette tip and a micropipette set to 10 µL. Use a new, sterile pipette tip for each colony to be picked.
3. Transfer each picked colony into an individual well of a 96-well plate containing 100 - 200 µL per well of liquid medium suitable for culturing CHO cells (e.g. ClonaCell™-CHO CD Liquid Medium). Gently resuspend each colony by pipetting up and down several times. The cells do not need to be in a single-cell suspension, but gently dispersing each colony will promote cell expansion.

   NOTE: Dispersal of the colonies may be performed using a multi-channel pipettor after all selected colonies have been transferred to the 96-well plate.
4. Incubate the 96-well plate at 37°C in 5% CO_2 and ≥ 95% humidity for 3 - 4 days, then screen the expanded cells using appropriate assays. Cultures that test positive may be expanded for further analysis, subcloning, or cryopreservation. Subcloning is recommended for generating stable, high-expressing clones.

References

Druz A et al. (2013) Stable inhibition of mmu-miR-466h-5p improves apoptosis resistance and protein production in CHO cells. Metab Eng 16: 87–94.

Wognum B & Lee T. (2013) Simultaneous cloning and selection of hybridomas and transfected cell lines in semisolid media. Methods Mol Biol 946: 133–49.

Young ARJ et al. (2013) Cell senescence as both a dynamic and a static phenotype. Methods Mol Biol 965: 1–13.

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