ClonaCell™ FLEX

Semi-solid methylcellulose-based medium for selecting and cloning cells

Catalog # 03818 45 mL

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Product Description

ClonaCell™ FLEX is a base methylcellulose medium that requires the addition of a 2X concentrate of the user's own liquid medium to create a custom semi-solid cloning medium. This allows the user to adapt an existing liquid cloning method to a customized semi-solid cloning method. ClonaCell™ FLEX is recommended for selection and cloning of suspension-adapted mammalian cells, including CHO cells and hybridomas. The medium is chemically defined, animal component-free, and protein-free. It does not contain L-glutamine, selection agents, or phenol red. ClonaCell™ FLEX is compatible with a wide range of selection systems, including dihydrofolate reductase (DHFR), glutamine synthetase (GS), and hypoxanthine, aminopterin, thymidine (HAT).

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.

Properties

Storage:	Store at -20°C.
Shelf Life:	Stable until expiry date (EXP) on label.
Contains:	• Methylcellulose • Water

Handling / Directions For Use

For optimal results, cells should be in logarithmic growth phase and viability should be greater than 90% at the time of plating.

NOTE: The plating efficiency in semi-solid media and the minimum antibiotic concentration required for selection of transfected cells varies between cell lines. For each cell line, the optimal plating density and antibiotic selection levels should be determined in titration experiments with nontransfected cells.

PREPARATION OF COMPLETE ClonaCell™ FLEX MEDIUM
The following example is for preparing 100 mL of complete ClonaCell™ FLEX medium. For other volumes, adjust accordingly.

1. Thaw bottle of ClonaCell™ FLEX at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix well.
   NOTE: Do not thaw ClonaCell™ FLEX in a 37°C water bath.
   NOTE: If ClonaCell™ FLEX is not used immediately, store at 2 - 8°C for up to 1 week. Alternatively, aliquot and store at -20°C. A 2X liquid medium concentrate and other additives may be added to the medium before or after aliquoting. Do not exceed the expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.
2. To bottle containing 45 mL of ClonaCell™ FLEX, add an equal volume of a 2X liquid medium concentrate that is appropriate for the cell line and selection system being used.
3. Add any other reagents required for growth and selection of the cells (e.g. L-Glutamine, Catalog #07100 or G418, Catalog #03812).
   NOTE: A total volume of 10 mL is available for the addition of supplements and cells, which should be prepared separately in 1X liquid medium.
4. Before adding cells, shake bottle vigorously to thoroughly mix additives. Allow medium to warm to room temperature (15 - 25°C).

5. Add cells to bottle and top up to 100 mL with 1X liquid medium if required. Mix well.
   NOTE: The final total volume of additives, supplements and cells in complete ClonaCell™ FLEX medium should not exceed 100 mL, as the viscosity may be too low at volumes > 100 mL. Low viscosity can result in diffuse colonies. If a higher viscosity is desired for the selection medium, the final volume may be reduced to < 100 mL.
   NOTE: It is recommended that several different plating densities be used, as transfection/plating efficiencies may vary between different cell lines and experiments.
6. Let the medium containing cells stand for 10 - 15 minutes to allow bubbles to dissipate.

CULTURING CELLS

1. Using a 12 mL syringe and a 16 gauge Blunt-End Needle (Catalog #28110), plate 9.5 mL of cell suspension into each of ten 100 mm Petri dishes. Alternatively, 1.5 - 2 mL of cell suspension medium may be dispensed into each well of eight 6-well plates.
   NOTE: Do not use a standard pipette to aliquot methylcellulose as the volume dispensed will not be accurate. The use of blunt-end needles for dispensing prevents needle-stick injuries.
2. Evenly distribute the cell suspension by gently tilting and rotating the plates. Incubate at 37°C in a humidified atmosphere containing 5% CO_2. If desired, the plates may be placed inside a plastic container (e.g. 245 mm x 245 mm Square Treated Tissue Culture Dishes, Catalog #27141) along with an open 100 mm Petri dish containing sterile distilled water. Ensure the lid of the storage container fits loosely to facilitate gas exchange.
3. Allow colonies to form for 10 - 14 days without disturbing the plates. The incubator should be well-humidified to prevent excessive evaporation.
4. Colonies will generally be macroscopically visible by day 12 - 14 of growth, but the optimal culture time will vary depending on the cell line and selection system used. Colonies may be picked and dispersed into the appropriate liquid growth medium for further expansion and testing.