AbeoClone™ (CHO TCSC Semi-sold Medium)

Transfected Cell Selection and Cloning

CATALOG#: ENZ-70010-0090 90mL/bottle

BACKGROUND

Liquid selection and cloning of transfected cells is often time-consuming, expensive and limiting in the number of clones that can be evaluated at one time. AbeoClone™ (CHO TCSC Semi-solid Medium) contains a proprietary blend of growth factors and pre-selected fetal bovine serum (FBS) in a methylcellulose base and is designed for supporting the growth of discrete colonies in a 3-dimensional matrix of a wide variety of cell types. In this semi-solid matrix, colonies are easily manually harvested using a sterile micropipette tip or with automated colony-picking systems. AbeoClone™ (CHO TCSC Semi-solid Medium) does not contain any selection agents and is optimized for the selection and cloning of a variety of cell lines including suspension-adapted lines such as CHO-S and hybridomas, in addition to some cell lines that grow adherently.

STABILITY AND STORAGE

Product stable at -20°C until expiration date as indicated on label. Avoid repeated freezing and thawing by storing aliquots at -20°C. Storage at 2-8°C is not recommended. Product is sterility tested.

DIRECTIONS FOR USE

1. Thaw AbeoClone™ (CHO TCSC Semi-solid Medium) at 2-8°C overnight or at room temperature (15-25°C). Mix well. The product should not be thawed at 37°C or higher temperatures.
2. Add IMDM, growth factors, selection agents and other supplements as desired along with cells to achieve a final volume of 100mL (i.e. liquid culture components+ cells+ semisolid medium =100 mL). The total volume of the liquid medium containing any supplements and cells should not exceed 10 mL as this will lower the viscosity of the semisolid medium resulting in “runny” colonies. *Note: If a higher viscosity is desired, the liquid medium volume may be reduced.*
3. Once culture components and/or cells are added to AbeoClone™ (CHO TCSC Semi-solid Medium), mix well by shaking the bottles vigorously then allowing the suspension to sit for 5-10 minutes to allow air bubbles to rise to the top.
4. Dispense the suspension into tubes or dishes using a syringe and 16-18 gauge blunt-end needle for safety reasons. The dispensed medium may be evenly distributed by gently rotating and tilting each dish. . Incubate the pated cells at 37^0C in a humidified, 5% CO_2 incubator. To prevent dehydration of the semisolid medium, place the dishes together in a separate plastic container such as a 500 cm^2 culture plate with an open

100mm culture dish containing sterile distilled water. Incubate at 37°C, 5% CO_2, in well-humidified incubator.

5. Do not disturb the plates for the first 10 days.
6. Approximately ten days after cells are plated in AbeoClone™ (CHO TCSC Semi-solid Medium), examine the plates for the presence of visible colonies. Colonies may be picked and dispersed into the appropriate liquid medium for further expansion and testing.

Further information can be found on **www.enzolifesciences.com**.

REFERENCES

1. Price, P.W., McKinney, E.C., Wang, Y., Sasser, L.E., Kandasamy, M.K., Matsuuchi, L., Milcarek, C., Deal, R.B., Culver, D.G., and Meagher, R.B. (2009). Engineered cell surface expression of membrane immunoglobulin as a means to identify monoclonal antibody-secreting hybridomas. J Immunol Methods 343, 28-41.
2. Köhler G and Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. Nature 256:495-497, 1975
3. Melchers F, Potter M and Warner NL. Lymphocyte hybridomas. Second workshop on "functional properties of tumours of T and B lymphocytes." Preface. Current Topics in Microbiology & Immunology 81:9-23, 1978
4. Kennett RH, McKearn TJ and Bechtol KB. Monoclonal antibodies. Plenum Press, New York 1980
5. Goding JW. Antibody production by hybridomas. J. Immunol. Methods 39:285-308, 1980
6. Sharon J, Morrison SL and Kabat EA. Detection of specific hybridoma clones by replica immunoadsorption of their secreted antibodies. Proc. Natl. Acad.Sci. 76:1420-1424, 1979
7. Pearson TW, Pinder M, Roelants G, Kar S, Lundin L, Mayor-Whithey KS and Hewett RS. Methods for derivation and detection of anti-parasite monoclonal antibodies. J. Immunol. Methods.34:141-154, 1980
8. Davis JM, Pennington JE, Kubler AM and Conscience JK. A simple, single-step technique for selecting and cloning hybridomas for the production of monoclonal antibodies. J. Immunol. Methods 50:161-171, 1982

Recommended for: One-step selection and cloning of transfected suspension-adapted and adherent cell lines including CHO-S, CHO-K1 and HEK-293.

Recloning of previously established suspension-adapted CHO cell lines

Accessory Products:
- Blunt-end 16 or 18 gauge needles
- 10 mL luer-lock disposable syringes
- 10 cm sterile plastic disposable Petri dishes (non-tissue culture treated)

Intended Use Statement: For Research Use Only. Not for Therapeutic or Diagnostic Use.

Contains:
- Methylcellulose
- Iscove's Modified Dulbecco's Medium (IMDM)
- Antibiotics/antimycotics
- Pre-selected serum
- Growth Factor Supplements

Does not Contain:
- Selective Agents

Product Type: Hybridoma generation & transfected cell selection

Application:
- Monoclonal cell line isolation and development
- Individual cells are physically separated by suspension in the viscous medium and are able to grow into discrete colonies that can be easily isolated
- Rare, high-producing clones are easily isolated compared with selection in liquid medium

Advantage: Compared with limited dilution cloning, semi-solid cloning is faster and requires fewer resources to isolate monoclonal cell lines.
Enables isolation of diverse clones with a variety of growth rates and productivities compared to selection in bulk liquid medium.

Area of Interest: Cell line development, Hybridoma generation, Pharmacology, toxicology, drug discovery

Cell Type: CHO

Medium Type: Methylcellulose-based

Manufactured by Abeome