



Semi-Solid Cloning with ClonaCell™-HY

Robust Colony Formation in ClonaCell™-HY Medium D

Monoclonal Antibody Development

Monoclonal antibodies (mAbs) are used as reagents for many applications, including laboratory research, immunological assay development and biopharmaceutical target validation and preclinical development. Generation of mAbs for these applications depends on the development of stable, high-producing hybridoma cell lines. Hybridoma development involves fusion of myeloma cells with spleen cells typically obtained from immunized mice, followed by selection of hybridomas by culturing them in a medium that promotes hybridoma growth and contains appropriate selection reagents. Cloning stable, high-producing cell lines from the polyclonal hybridoma pool obtained after fusion is a necessary and often laborious step in monoclonal antibody development. Hybridomas can be cloned using several methods, including limiting dilution cloning, fluorescence-activated cell sorting (FACS) and semi-solid cloning in a methylcellulose-based medium.

Semi-Solid Cloning in Methylcellulose-Based Medium

During semi-solid cloning, individual cells are immobilized within a viscous medium and grow into visibly discrete, monoclonal colonies (Figure 1). Picking these discrete colonies enables isolation of diverse clones with a high probability of monoclonality. Using semi-solid cloning after hybridoma fusion can streamline the cell line development process as it requires fewer plates and less medium to isolate a greater number of clones with a high probability of monoclonality than selection and cloning in liquid medium by limiting dilution. Additionally, both fast- and slow-growing clones form discrete colonies during semi-solid cloning, making it easy to isolate and screen a diverse range of clones. This diversity increases the probability of finding rare clones with desirable characteristics. Semi-solid cloning can also be used to re-clone the most desirable cells in an existing hybridoma cell line where the production properties have deteriorated over time.

ClonaCell™-HY Methylcellulose-Based Cloning Media

ClonaCell™-HY Medium D and ClonaCell™-HY Medium D Without HAT are high-performance, methylcellulose-based media for semi-solid cloning of hybridomas. ClonaCell™-HY Medium D contains hypoxanthine, aminopterin and thymidine (HAT) selection

Find the Right Clone, Faster! Benefits of Semi-Solid Cloning with ClonaCell™-HY Medium D:

- Isolate a large number of unique hybridomas, each with a high probability of monoclonality
- Obtain robust growth of mouse and human hybridomas, ensuring more fusion products survive to form colonies
- Isolate highly diverse clones, making it easier to find clones with rare characteristics

PRODUCT: ClonaCell™-HY Medium D
CATALOG #: 03804 90 mL
(for 100 mL complete medium)

reagents, while ClonaCell™-HY Medium D Without HAT can be used for cloning with other selection systems. When compared with a methylcellulose-based medium for hybridoma and myeloma cells from another supplier*, ClonaCell™-HY Medium D supports the growth of significantly more hybridomas (Figures 1 and 2). In these experiments, a greater number of hybridomas survived to form colonies in ClonaCell™-HY medium, enabling isolation of more hybridomas per mouse and helping to ensure that valuable clones are not lost during the cloning process. The total number of hybridomas and positive clones obtained after selection and cloning by semi-solid cloning in ClonaCell™-HY Medium D was similar to that obtained by limiting dilution cloning in a comparable liquid medium (ClonaCell™-HY Liquid HAT Selection Medium, Catalog #03831). ClonaCell™-HY methylcellulose-based media are compatible with semi-solid cloning followed by manual colony isolation, as well as automated colony isolation using instruments such as the ClonaCell™ EasyPick and the ClonePix™ 2-FL (Molecular Devices®).



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Robust Colony Formation in ClonaCell™-HY Medium D

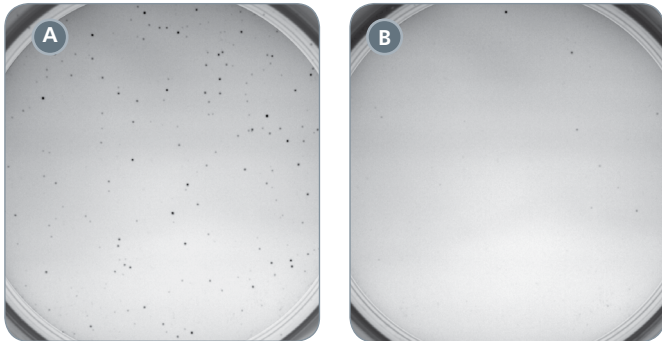


Figure 1. Semi-solid cloning of hybridoma cells yields more colonies in ClonaCell™-HY Medium D than in a competitor medium*

Spleen cells from a BALB/c mouse immunized with the protein antigen ovalbumin were fused with SP2/0 myeloma cells and plated according to the standard protocol described in the ClonaCell™-HY Technical Manual (Document #28411; Fusion Method A). An equal number of cells were suspended in 10 mL (A) ClonaCell™-HY Medium D or (B) a methylcellulose-based medium from a different supplier* prepared according to the manufacturer's instructions. The cells were plated in 10 cm culture dishes and cultured in a humidified incubator at 37°C and 5% CO₂ for 14 days, after which time colonies were photographed and counted using the automated ClonaCell™ EasyPick instrument (Catalog #30000).

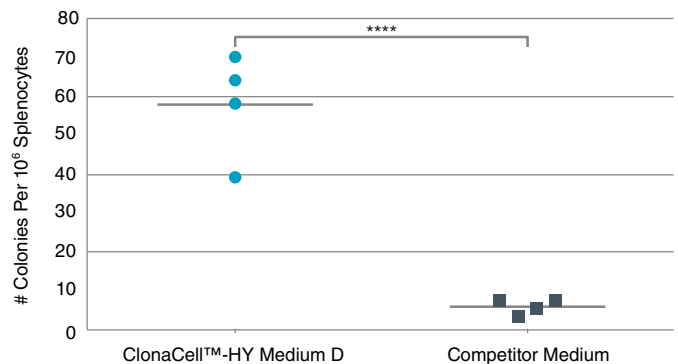


Figure 2. Number of colonies for hybridoma fusions plated in ClonaCell™-HY Medium D vs. competitor medium*

Spleen cells from four ovalbumin-immunized BALB/c mice were fused with SP2/0 myeloma cells and plated according to the standard protocol described in the ClonaCell™-HY Technical Manual (Document #28411; Fusion Method A). Cells from each mouse were plated at four plating densities in ClonaCell™-HY Medium D or a methylcellulose-based medium from a different supplier*. Colonies were counted using the automated ClonaCell™ EasyPick platform after 14 days of culture. The dot plot shows the average number of colonies per 10⁶ splenocytes for each of the four immunized mice. Horizontal lines indicate the mean value for each medium. The differences between the media were highly significant (Poisson regression: $p < 0.0001$).



VIDEO

Learn more about the process and benefits of semi-solid cloning:
www.stemcell.com/Try_SSC

PRODUCT	CATALOG #	DESCRIPTION	RECOMMENDED FOR
ClonaCell™-HY Medium D	03804	Methylcellulose-based hybridoma selection and cloning medium containing HAT	Semi-solid cloning and HAT selection of hybridomas
ClonaCell™-HY Medium D without HAT	03810	Methylcellulose-based hybridoma cloning medium containing no selection agents	Semi-solid cloning of hybridomas using a selection system other than HAT
ClonaCell™-HY Kit	03800	Complete cell culture media kit for hybridoma fusion and cloning	Generating monoclonal hybridomas from myeloma cells and splenocytes using semi-solid cloning with HAT selection
ClonaCell™-HY Medium E	03805	Hybridoma growth medium	Supporting robust growth of mouse and human hybridoma cultures
ClonaCell™-HY Medium A	03801	Myeloma growth medium	Supporting growth of myeloma cultures and long-term maintenance of hybridoma cultures

HAT = hypoxanthine-aminopterin-thymidine

*CloneMedia (Molecular Devices®, catalog #K8610), supplemented with equivalent concentrations of HAT as ClonaCell™-HY Medium D (Catalog #03804).

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