

TECHNICAL NOTE

ClonaCell™-HY 96-Well Plate Selection and Cloning

Background

Conventional methods to select and clone monoclonal antibody-producing hybridomas involve multiple dilution steps in liquid medium. STEMCELL Technologies provides a methylcellulose-based medium, **ClonaCell™-HY Medium D**, which combines the hybridoma selection and cloning steps, reducing the overall time necessary to produce monoclonal antibodies by up to 19 days. Using the standard protocol for semi-solid cloning of hybridomas in ClonaCell™-HY media, the fused cells (i.e. fused lymphocyte/myeloma hybrids) are suspended in selective methylcellulose-based medium and incubated in 10-cm plates. Hybridomas grow to form colonies which can be picked from the semi-solid medium after 10 - 14 days of incubation. Colonies are transferred to wells of a 96-well plate and cultured in liquid medium prior to screening supernatants for positive clones. To learn more about this technique, visit: www.stemcell.com/cloning-with-clonacell.

The following technical note describes the use of semi-solid methylcellulose-based medium (**ClonaCell™-HY Medium D**) and 96-well plates to reduce the need to harvest and expand large numbers of hybridoma colonies before screening (Figure 1). A fused cell suspension mixed with **ClonaCell™-HY Medium D** is plated directly into individual wells of a 96-well plate. Hybridomas grow in the viscous medium as discrete colonies and secrete antibodies into the surrounding medium. Liquid medium is layered over the semi-solid medium and the secreted antibodies diffuse into the liquid medium. The liquid medium is then harvested and screened for specific antibodies. Using this method, colonies can be tested for secretion of specific antibodies without the need to harvest and expand every colony first. This protocol eliminates picking and expansion of non-producing colonies, resulting in considerable time and labour savings. This protocol is designed to reduce screening when picking colonies manually. It is not compatible with automated colony picking with the ClonaCell™ EasyPick instrument (Catalog # 30000).

Protocol

It is recommended that a fresh vial of myeloma cells be thawed and cultured at least one week prior to the expected fusion date. Ensure myeloma cells are mycoplasma-free. Ideally, the cells used for fusion should be in their logarithmic growth phase.

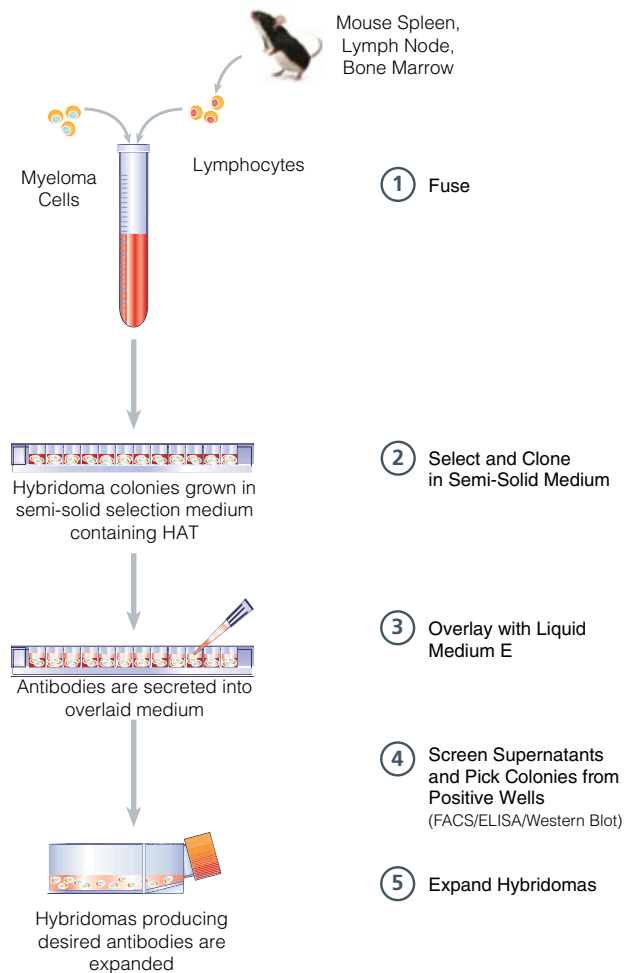
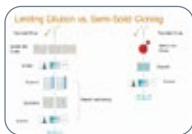


FIGURE 1. ClonaCell™-HY 96-Well Procedure



WEBINAR

A Smarter Way to Clone

www.stemcell.com/cloning-with-clonacell



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- On the day of fusion, place the hybridoma semi-solid cloning medium, **ClonaCell™-HY Medium D** (Catalog #03804), at 2 - 8°C to thaw overnight. Do not place medium in a water bath to thaw.
- Perform fusion of myeloma cells and lymphocytes to yield between 10 - 80 million fused hybridoma cells. For a more detailed fusion protocol, please refer to the ClonaCell™-HY Technical Manual (available at www.stemcell.com).
- Incubate the fused cells in the recovery medium, **ClonaCell™-HY Medium C** (Catalog #03803), at 37°C in a humidified, 5% CO₂ incubator for 16 - 24 hours.
- On the day after fusion, vigorously shake the thawed Medium D to mix the contents of the bottle and warm to 37°C.
- Determine the optimal number of cells to plate per well to arrive at one colony per well. We recommend a range of 10,000 - 80,000 cells/well. If you already have experience with hybridoma selection in liquid HAT medium, plate the same number of cells per well in the semi-solid medium as you would in liquid medium.
- Resuspend the cells in Medium C for a total volume of 10 mL. It is critical not to exceed 10 mL final volume. If you plan to add additional cytokines or growth factors to Medium D, include them with the cells in a total 10 mL volume.
- Combine the 10 mL fused cell suspension with 90 mL Medium D. Mix thoroughly and let sit for 15 minutes to allow the bubbles to rise to the surface.
- Using either a multi-channel pipettor and sterile wide-bore pipette tips or a repeat pipettor and sterile syringe, dispense 60 - 80 µL of Medium D into each well of a 96-well plate. This will yield between 12 and 16 plates depending on the volume plated. Medium D is a viscous solution and therefore difficult to pipette accurately; however, it is not critical to dispense exactly the same volume into each well.
- Incubate the plates at 37°C in a humidified, 5% CO₂ incubator. The incubator should be well-humidified to prevent excessive evaporation. To prevent dehydration, the plates may be placed inside a plastic container that allows proper gas exchange (e.g. 245 mm square treated tissue culture dishes; Catalog #38039/100-0084) along with an open Petri dish containing sterile water.
- Following 8 days of undisturbed incubation, examine wells for the presence of colonies by eye or microscope and gently overlay 150 µL of pre-warmed (37°C) hybridoma growth medium, **ClonaCell™-HY Medium E** (Catalog #03805), onto the semi-solid medium in each well containing colonies. Alternatively, all wells may be overlaid with 150 µL of pre-warmed Medium E, regardless of the presence of colonies, and analysis is then performed on all wells.
- Incubate plates at 37°C in a humidified, 5% CO₂ incubator for an additional 2 - 4 days. The overlay incubation time may be increased further to ensure the detection of low-expressing hybridomas.
- Carefully remove a maximum of 100 µL of the overlaid Medium E without disturbing the colonies in the semi-solid medium. Test the supernatants for specific antibodies using an assay system appropriate for the antigen involved (e.g. ELISA, flow cytometry, western blotting).
- The contents of wells that tested positive for antibodies against the antigen of interest should be gently resuspended and transferred to a single well of a 24-well plate containing 1 mL of Medium E, to expand the hybridomas. If a well contains more than one colony, it may be possible to harvest these clones separately and transfer them to individual wells for expansion and retesting. If wells contain more than one colony and harvesting of individual colonies is not possible, the hybridomas need to be recloned either immediately after harvesting or after a brief 1 - 2 days recovery and expansion period in Medium E. Recloning is not necessary for positive clones which can be harvested independently as these hybridomas have a high probability of being monoclonal. However, it is useful to reclone these hybridomas when selecting for stable, high-producing subclones.

For additional details on hybridoma generation please refer to the Technical Manual for ClonaCell™-HY Hybridoma Cloning Kit (Manual Document #28411), available at www.stemcell.com.

Time Comparison of Traditional, ClonaCell™-HY, and ClonaCell™-HY 96-Well Protocols for Hybridoma Generation

LIQUID MEDIA	TIME (DAYS)*	CLONACELL™-HY	TIME (DAYS)*	CLONACELL™-HY 96-WELL PROTOCOL	TIME (DAYS)*
Fuse	1	Fuse	1	Fuse	1
Selection and Cloning by Limiting Dilution	14	Selection and Cloning in 10 cm Dish	10-14	Selection and Cloning in 96-Well Plates	8
Screening	1	Colony Isolation	1	Overlay with Medium E	2-4
Subcloning by Limiting Dilution	14	Expansion	2-4	Screening and Isolating Colonies (Monoclonal Hybridomas)	1
Screening	1	Screening	1		
Total Days	31	Total Days	15-21	Total Days	12-14

*Estimated times will vary depending on volume of work. We recommend a round of subcloning to obtain stable, high-producing hybridoma cell lines.