TOOLS TO INCREASE THE EFFICIENCY OF YOUR HYBRIDOMA WORKFLOW

Work Smart with EasySep™ and ClonaCell™

Hybridoma technology has enabled the screening and production of monoclonal antibodies (mAbs). The workflow to generate hybridomas is arduous, and has seen few improvements since its inception. Nevertheless, hybridomas have been used to generate mAbs for numerous downstream applications in laboratory research, immunological assay development, and preclinical development. To improve the process for generating hybridomas, STEMCELL Technologies has developed products that increase efficiency at key stages of the workflow.

The EasySep™ Mouse CD138 Positive Selection Kit (Catalog #18957) can be used for the upstream enrichment of antibody-secreting cells (Figures 1 and 2), with sufficient recovery to be used in fusions (Figure 3). Fusions with CD138$^+$ cells, as compared to total splenocytes, produced a higher percentage of antibody-secreting hybridomas (Figure 3A). Additionally, fusions using CD138$^+$ cells generated a higher proportion of hybridomas producing antigen-specific antibodies (Figure 3B). The survival of hybridomas during screening, cloning, and expansion is important. When compared to DMEM + 10% FBS, ClonaCell™-HY Medium E (Catalog #03805) and ClonaCell™-HY AOF Expansion & Cloning Medium (Catalog #03835) improve clonal survival, allowing antibody screening and cloning to be reliably performed in either serum-containing or serum-free media (Figure 4).

Why Use EasySep™ and ClonaCell™ for Your Hybridoma Workflow?

FLEXIBLE. Easily integrate products into existing workflows.

EFFICIENT. Quickly isolate CD138$^+$ cells with ease, and selectively fuse antibody-producing cells.

SIMPLE AND CONVENIENT. Simplify time-consuming steps in hybridoma development.

VERSATILE. Supports single-cell cloning applications, hybridoma expansion, and antibody production.

RELIABLE. Rigorous performance testing ensures lot-to-lot reproducibility.

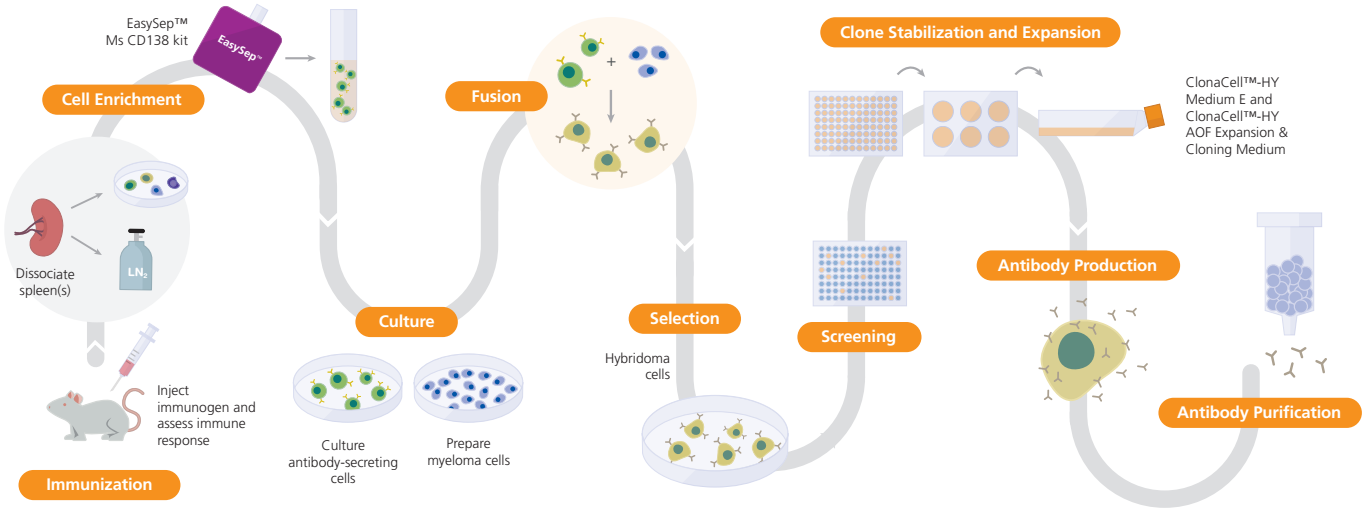

Figure 1. Hybridoma Workflow Using EasySep™ and ClonaCell™

The dissociated spleen (or other lymphoid tissue) from an immunized animal is used in fusion reactions with myeloma cells to generate hybridomas. The EasySep™ Mouse CD138 Kit enriches for antibody-secreting cells prior to fusion. Using the antibody-secreting subset of cells for fusion reduces the number of clones to be screened and increases antigen-specific hit rates. Hybridomas are selected for antibody secretion and target specificity, and the desired clones are stabilized over time using ClonaCell™-HY Medium E or ClonaCell™-HY AOF Expansion & Cloning Medium. These media are designed to support survival and growth during antibody screening and the verification of monoclonality. A stabilized clone can be used to produce antibodies, which are used in functional verification. Antibody production in a serum-free medium such as ClonaCell™-HY AOF Expansion & Cloning Medium allows antibodies to be analyzed and/or purified in the absence of contaminating (serum-derived) immunoglobulin.

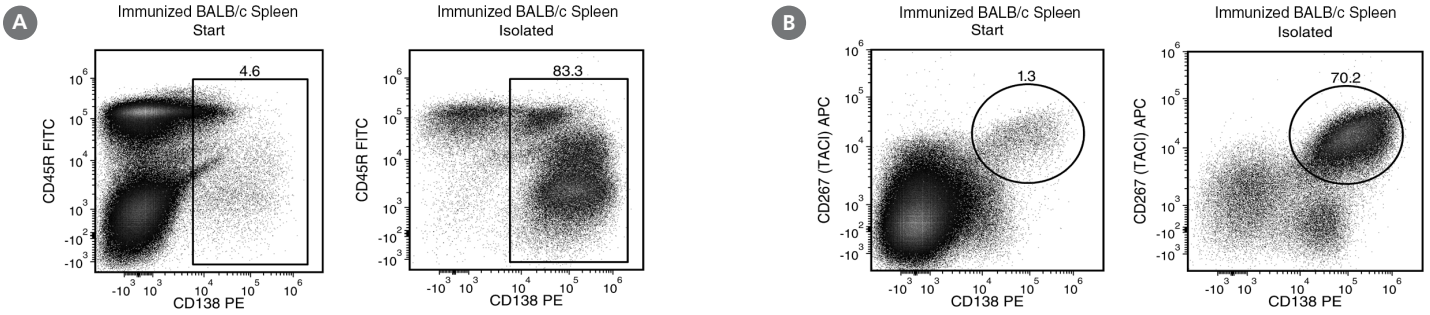

Figure 2. Purity of Total CD138⁺ cells and CD138⁺CD267 (TACI)⁺ Plasma Cells/Blasts Before and After Being Isolated by EasySep™

CD138⁺ cells were isolated from immunized BALB/c mouse splenocytes with the EasySep™ Mouse CD138 Positive Selection Kit. The samples were analyzed by flow cytometry before and after cells were isolated. (A) The total CD138⁺ cell content of the isolated cells is typically 81.5 ± 4.9% (mean ± SD). Here, the purities of the start and final isolated fractions are 4.6% and 83.3%, respectively. (B) The CD138⁺CD267 (TACI)⁺ plasma cell/blast content of the isolated cells is typically 68.5 ± 11.3% (mean ± SD). Here, the purities of the start and final isolated fractions are 1.3% and 70.2%, respectively.

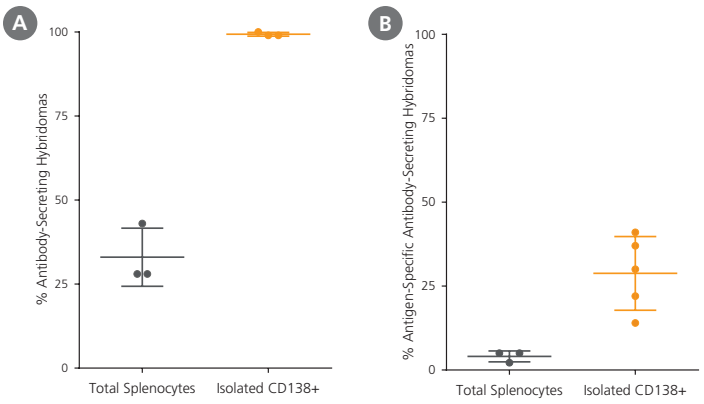

Figure 3. Antibody-Secreting and Antigen-Specific Hit Rates for Hybridomas Generated Using Total Splenocytes or Isolated CD138⁺ Cells and Cultured in ClonaCell™-HY Medium D

Total splenocytes or CD138⁺ cells isolated from mice immunized with various antigens were fused with Sp2/0 mouse myeloma cells that had been cultured in ClonaCell™-HY Medium A (Catalog #03801) and washed with ClonaCell™-HY Medium B (Catalog #03802). Fused cells were cultured in ClonaCell™-HY Medium C (Catalog #03803) to promote recovery and plated in ClonaCell™-HY Medium D (Catalog #03804), a semi-solid medium. (A) The % antibody-secreting hybridomas, as determined by ELISA, is shown for hybridomas generated using total splenocytes (33.0 ± 8.7%; mean ±SD) and isolated CD138⁺ cells (99.3 ± 0.6%). (B) The % antigen-specific hit rate, as determined by ELISA, is shown for hybridomas generated using total splenocytes (4.1 ± 1.6%; mean ±SD) and isolated CD138⁺ cells (28.8 ± 11.0%).

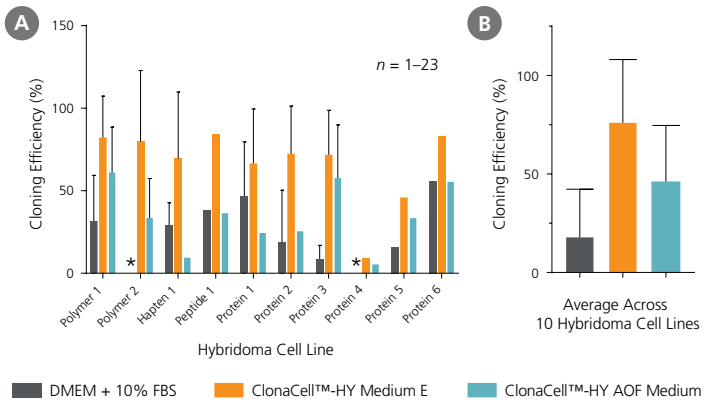

Figure 4. Cloning Efficiencies for Ten Hybridoma Cell Lines Subcloned in Serum-Containing and Serum-Free/Animal Origin-Free Cell Culture Media

Hybridoma clones secreting antibodies specific for a variety of antigens were subcloned by limiting dilution in either DMEM + 10% FBS, the serum-containing ClonaCell™-HY Medium E, or the serum-free (animal origin-free; AOF) ClonaCell™- HY AOF Expansion & Cloning Medium. After incubation for 12 - 14 days (37°C, 5% CO2), the plates were examined under a microscope and assessed for growth. (A) Cloning efficiency, as estimated by Poisson statistics using the ELDA method described by Hu & Smith, (2009, J Immunol Meth, 347: 70 - 78). (B) Average cloning efficiency obtained for the ten hybridoma cell lines in each cell culture medium. *Cloning efficiencies of 0% were observed for DMEM + 10% FBS in 18 separate experiments for Polymer 2 and in 1 experiment for Protein 4.

Product	Size	Catalog #
EasySep™ Mouse CD138 Positive Selection Kit	1 kit	18957
EasySep™ Release Mouse CD138 Positive Selection Kit	1 kit	100-0601
ClonaCell™-HY Medium A	500 mL	03801
ClonaCell™-HY Medium B	500 mL	03802
ClonaCell™-HY Medium C	100 mL	03803
ClonaCell™-HY Medium D	90 mL	03804
ClonaCell™-HY Medium E	500 mL	03805
ClonaCell™-HY AOF Expansion and Cloning Medium	500 mL	03835
Anti-Mouse CD45R Antibody, Clone RA3-6B2	Various	60019
Anti-Mouse CD138 (Syndecan-1) Antibody, Clone 281-2	Various	60035
Anti-Mouse CD267 (TACI) Antibody, Clone 8F10	Various	60116