Benchmark

Manufacture of Pure Monoclonal Antibodies by Heterogeneous Culture without Downstream Purification

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The manufacture of monoclonal antibodies (mABs) is expensive and requires specialized equipment and technical skills in tissue culture. Hybridoma cells (3) *in vitro* require the essential additive fetal calf serum (FCS) (4) to ensure adequate growth. FCS, however, contains proteins such as transferrin (75 kDa) and albumin (66 kDa) that contaminate the mAB supernatant harvests. Downstream purification (2) ensures the removal of contaminating proteins but is time consuming, reduces the total yields of mAB product (7), and requires

specialized equipment. Harsh purification procedures may also damage or modify the mAB product (6).

A disposable, heterogeneous cell cultivation system, the CELLLine® 1000 (Integra Bioscience, Wallisellen, Switzerland) utilizes the principle of membrane diffusion (1) to allow nutrient access to cells growing in *in vitro* culture. The advantage over the homogeneous static equivalent method is that the hybridoma cells and their mAB product remain trapped and concentrated in a small 20-mL compartment at the base of the 1000-mL vessel. Nutrients diffuse into the cell compartment across a semi-permeable membrane made of regenerated cellulose (8 µm) with a 10 000 molecular weight cut off. Oxygen and carbon dioxide diffuse across a

silicone-based gas membrane at the base of the compartment. The cells remain viable and grow in high density in the cell compartment. No monitoring devices are required, and the cells can be examined microscopically without removal from the vessel. According to the supplier, mAB manufacture in the CELLLine 1000 can reduce costs by 40%, but harvested mAB product still requires downstream purification (6) to remove contaminating products from FCS.

A novel modification of the CELLLine 1000 is described (Figure 1) that yields "pure" mAB product within seven days with no need for downstream purification. FCS is placed above the semi-permeable membrane and not in direct contact with the hy-

Figure 1. Schematic representation of the heterogeneous culture system. (A) FCS in the cell compartment (recommended by the supplier). (B) FCS above the semi-permeable membrane.

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bridoma cells. Although FCS is not placed within the cell compartment, trapped cells are still able to utilize essential nutrients from the FCS that diffuse across the semi-permeable membrane. The RFT-4 hybridoma cell line used in this study is not conditioned to a serum-free environment and requires a minimum of 5% FCS to ensure growth. This confirms that essential nutrients from FCS can diffuse across the semi-permeable membrane, and the modification to the CELLLine 1000 would be suitable to other hybridoma cell lines reliant on FCS supplementation.

The hybridoma cells were cultured in standard 75-mL static homogeneous culture flasks in RPMI 1640 media containing 10% FCS. At log phase, 2×10^7 hybridoma cells were centrifuged ($200 \times g$ for 10 min) and washed in RPMI 1640 media to remove the FCS. These cells were resuspended in 20 mL RPMI 1640 media with no FCS and planted into the cell compartment of the CELLLine 1000 vessel. The compartment above the semi-permeable membrane was filled with 1000 mL RPMI 1640 containing 10% FCS with 1% penicillin/streptomycin antibiotics. The vessels were incubated at 37°C in the presence of 5% CO₂ and 80% humidity for seven days before harvesting. On day 7, the entire cell compartment content was harvested and centrifuged to discard the cells and cell debris. The supernatant with the mAB product was sterile filtered (0.22 µm), and the concentration was determined by murine ELISA (Roche Molecular Biochemicals, Mannheim, Germany). Every seven days after each harvest, 1000 mL media with 10% FCS was replaced above the semi-permeable membrane, and new log-phase hybridoma cells were planted into the cell compartment. When not in use, the vessels were rinsed with sterile saline and stored in sterile saline at 4°C for further use.

Compared with the recommended method, the described modification requires a longer incubation period (seven days instead of three) and eight times more FCS. However, the harvested supernatant yielded pure mAB without further downstream purification. This is best illustrated by protein electrophoresis (5) (Figure 2), showing the reduced mAB product clearly separated

into immunoglobulin heavy chains (50 kDa) and light chains (25 kDa), at a concentration of 0.1 mg/mL. Culturing the hybridoma cells according to the manufacturer's instructions produced a more concentrated mAB product (0.5 mg/mL) but required downstream purification. Protein G affinity chromatography was used for the latter to obtain a product of comparable purity.

The RFT-4 hybridoma cell line produces murine mABs against the CD4 antigen on T-cell lymphocytes. This CD4 mAB is fluorochrome labeled with R-phycoerythrin and used in flow cytometry (data not shown) for research projects to enumerate CD4 T cells to measure disease progression in HIV/AIDS (8). The described modification to the CELLLine 1000 is best suited for mAB quantities required for research applications and can facilitate pure mAB production within seven days. Further, this novel application can

be especially useful when tissue culture laboratory space, in-line monitoring, and a clean air environment is not available and when tissue culture expertise may be a limiting factor.

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Figure 2. 10% SDS-PAGE of reduced mAB harvests. Lane 1, molecular weight markers. Lane 2, mAB product produced from hybridoma cells, using the modification of the CELLLine 1000 (with no FCS in the cell compartment), showing a pure product of 25 kDa light chain and 50 kDa heavy chain. Lane 3, mAB product from hybridoma cells cultured in the CELLLine 1000 according to the suppliers instruction (with FCS in the cell compartment), showing the presence of the contaminating proteins (including transferrin, 75 kDa and albumin, 66 kDa). Lane 4, downstream purification by protein G affinity chromatography (Pierce Chemical, Rockford, IL, USA) of IgG mAB from a harvest as in lane 3.