



Title: *Hybridoma Media Preparation: SP2/0-conditioned Medium*

No: RTLP-GL-Ab-7

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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|------------------------|---------------------------|----------------------------|----------------------|
| •Lab coat | • Pen/Strep Solution | •Phosphate | •Pipettes |
| •Gloves | •L-Glutamine 200mM | Buffered Saline | •Pipetteman |
| •DMEM-10 (RTLP-GL-Ab-6 | •Fetal Bovine Serum (FBS) | (PBS) | •Pipette Aid |
| •T75 flask | •50 mL tubes | •DMEM | •Pipetteman tips |
| •-20°C Storage | •Centrifuge | •Sterile laminar flow hood | •37°C incubator |
| | | | •0.22 um filter unit |

Procedure:

1. Inoculate a T75 flask containing DMEM-10 with 1×10^6 SP2/0 cells.
2. Pipette 60 mL of this into a T75 flask and incubate at 37°C. Monitor the growth of cells- by day 6, the cells should be at a cell density of $\sim 9.0 \times 10^5$ cells/mL.
3. Harvest cells and medium and place into 50 mL conical centrifuge tubes.
4. Pellet cells at 2,000 rpm (900 x g) for 20 minutes.
5. Pour supernatant fluid into sterile tubes and freeze at -20°C.
6. Thaw supernatant and filter sterilize through a 0.22 um filter.

Author	Management Approval/Date	Effective Date