

**SOP: M021**

**Preparation of Complete DMEM Media for Hybridoma Growth**

**Materials and Reagents:**

1. Antibiotic-Antimycotic 100x liquid (Invitrogen #15240)
2.  $\beta$ -Mercaptoethanol 1000x liquid (Invitrogen #21985)
3. DMEM/F12 + Glutamax + NaHCO<sub>3</sub> + Sodium Pyruvate (Invitrogen #10565)
4. Heat inactivated FBS, triple 0.2  $\mu$ m filtered (Atlas)
5. Tissue culture hood (SOP #SP041)
6. 1 L Nalgene 0.2  $\mu$ m filter
7. Serological pipettes (1, 5 and 50 ml)

**Protocol:**

1. \_\_\_\_\_ Prepare biosafety cabinet for work under sterile conditions (See SOP: SP041)
2. \_\_\_\_\_ Add 1 ml of 1000x  $\beta$ -Mercaptoethanol and 5 ml of 100x Antibiotic-Antimycotic to 500 ml DMEM/F12 + Glutamax + NaHCO<sub>3</sub> + Sodium pyruvate.
3. \_\_\_\_\_ Transfer medium to the top part of 1 L 0.2  $\mu$ m PES Nalgene filter and connect to the vacuum. Add 100 ml of heat inactivated FBS for 10% (or 200 ml of FBS if 20% DMEM is specified in the protocol). Filter the complete media (Note 1).
4. \_\_\_\_\_ Store at 4C for up to 2 months (note 2).

**Notes:**

1. After FBS has been added, the medium is considered “complete”.
2. Antibiotics have very short half-lives, meaning that they need to be replenished frequently (table 1).

Antibiotic/Antimycotic	Target	Half-life at 37°C	Mechanism of action
Penicillin G sodium	Gram positive bacteria	2 days	Cell wall inhibitor
Streptomycin sulfate	Gram negative bacteria	4 days	Inhibits translation in 30S subunit
Amphotericin B	Fungi, yeast	4 days	Alters membrane permeability

**Table 1 – Half-lives of Antibiotics and Antimycotics used in complete DMEM (Harlow and Lane).**

**References:**

*Antibodies: A Laboratory Manual*. Ed Harlow and David Lane. Cold Spring Harbor Laboratory, New York. 1988. pp. 247-281.

Kohler, G., and Milstein, C. *Nature* 256, 495-497. August 7, 1975. Continuous Cultures of Fused Cells Secreting Antibody of Predefined Specificity.

[http://www.piercenet.com/files/1601975\\_AntibodyHB\\_Version2\\_INTL.pdf](http://www.piercenet.com/files/1601975_AntibodyHB_Version2_INTL.pdf)

Wayne M. Yokoyama, Michelle Christensen, Gary Dos Santos, and Diane Miller. *Current Protocols in Immunology*. UNIT 2.5 Production of Monoclonal Antibodies. Published Online: September 1, 2006.