SOP: M029
Modified: 7/14/22 KE

Preparation of Complete RPMI Media for Hybridoma Cell Growth

Materials and Reagents:

1. RPMI-1640 (ATCC Modified), sterile-filtered, 500 mL bottle (ThermoFisher Cat # A1049101)
2. L-glutamine, 200mM (100X), 100 mL bottle (Fisher Scientific Cat # 25030081) -- ***OPTIONAL***
3. Antibiotic-Antimycotic 100X liquid, 100 mL bottle (Fisher Scientific Cat # 15240-062)
4. 2-Mercaptoethanol 1000X liquid, 50mL bottle (Life Technologies Corporation Cat # 21985-023)
5. Fetal Bovine Serum, Not heat inactivated (Sigma Cat# F2442-500mL, also available through PMF/ARCBIO)
6. HAT media supplement, 50X, 100 mL bottle (Gibco, Fisher Scientific Cat # 21060017)
7. HT media supplement, 100X, 50 mL bottle (Gibco, ThermoFisher Cat # 11067-030)
8. 500 mL or 1 L 0.22 µm sterile filter units, PES membranes
9. Serological pipets (assorted volumes)
10. P1000 pipet with ***filter*** tips
11. Automated pipetman
12. 2.5 % Vesphene (or other disinfectant of choice)
13. 70% Ethanol
14. Tissue culture biosafety cabinet

Protocol:

1._____ Prepare biosafety cabinet (BSC) for work under aseptic conditions (wipe down cabinet surface with 2.5% Vesphene (or disinfectant of choice) and then 70% ethanol. 70% isopropanol may be used if fungal contamination is of concern.

2._____ Prepare 500mL or 1L of media as follows (Notes 1-2 & 7) and mix well:

Component	Qty/1 L IMDM	Qty/500 mL IMDM	Final Concentration
RPMI-1640	Q.S. to 1L	Q.S. to 500 mL	N/A
*Antibiotic-antimycotic (100X)	10 mL	5 mL	1X
*FBS	100 mL/200 mL	50 mL/100 mL	10%/20%
*2-Mercaptoethanol (1000X)	1 mL	0.5 mL	1X
**L-glutamine, 200 mM (100X)	10 mL	5 mL	2 mM (1X)
HT (100X)	10 mL	5 mL	1X

Indicates hazardous substance (complete media must go in hazardous waste). See document**: Hybridoma Media Haz Waste_List of Components in "MAB_HYB_SOPs" folder to generate hazardous waste labels for complete media.*

***Optional supplement to add, NOT a hazardous reagent*

See Note 7 for information on HAT and HT supplements

3._____ In the BSC, transfer media to a 500 mL or 1 L 0.22 µm sterile filter unit (PES membrane) and connect to the vacuum. Filter the complete media (Note 3).

4._____ Aliquot media into smaller volumes (Note 4).

5._____ Store media at 4°C for up to 1 month (Notes 5 & 6).

Notes:

1. After FBS has been added, the medium is considered "complete".
2. Add up volume of supplements (reagents to be added to media) and remove the calculated volume from the 500 mL base media. Keep the "incomplete" base media in a sterile storage container for future use, if needed. This incomplete media can be dumped down the drain, if NOT hazardous waste.
3. Media can be filtered using a benchtop vacuum. However, make sure that the filter unit is sealed and not opened outside of BSC.
4. Aliquoting media allows for ease of warming up and saves media from contamination or becoming too alkaline (red color) due to repeated/constant use of big storage bottle.
5. Antibiotics have very short half-lives, meaning that they need to be replenished frequently (at least once every week).

   Table 1 – Half-lives of Antibiotics and Antimycotics (Harlow and Lane).

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

6. L-glutamine supplement should be replenished in the media after one month at 4°C and one week at 37°C.
7. For establishing hybridoma cell lines (fusion, parentals), add HAT supplement to a 1X concentration to the complete IMDM media with 20% FBS above. When parental hybridoma lines have been established, cells should be weaned off the HAT supplement. Parentals can be frozen in HAT media, but ideally weaned off and frozen in HT media (See SOP: AB104.6). Parentals should be thawed and grown in complete IMDM media, 20% FBS, with 1X HT (and all other additional supplements provided in this IMDM media SOP).

   Subcloned hybridomas (*established hybridoma cell lines*) should be grown with the 1X HT supplement, unless otherwise noted for a particular cell line. 10% or 20% FBS can be used for *established hybridoma cell line* growth (generally thaw/grow lines in 10% FBS – more cost effective and they grow slower).

Component	Qty/1 L IMDM	Qty/500 mL IMDM	Final Concentration
HAT (50X)	20 mL	10 mL	1X

Tips:

- To mix media, inverting the bottle is sufficient. Too vigorous of mixing creates bubbles (FBS).

References:

1. Antibodies: A Laboratory Manual. Ed Harlow and David Lane. Cold Spring Harbor Laboratory, New York. 1988. pp. 247-281.
2. Kohler, G., and Milstein, C. Nature 256, 495-497. August 7, 1975. Continuous Cultures of Fused Cells Secreting Antibody of Predefined Specificity.
3. 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.