

SOP:M012

Preparation of Complete RPMI Media

Materials and Reagents:

1. RPMI 1640 medium with L-glutamine, without sodium bicarbonate (Invitrogen 11875-093)
2. Fetal Calf Serum (FCS)
3. 2-mercaptoethanol
4. Dextrose (Sigma G-7021)
5. Essential Amino Acids 50X (Invitrogen 11130-051)
6. Non-essential Amino Acids 100X (Invitrogen 11140-050)
7. Sodium pyruvate 100X (Invitrogen 11360-070)
8. Sodium bicarbonate (Sigma S-5761)
9. Streptomycin/Penicillin 100X (Invitrogen 15140-122)
10. Nalgene filter unit, 0.2 μ m
11. 50ml falcon centrifuge tubes
12. Sterile roller bottle
13. 10N NaOH
14. Tissue culture hood
15. 15ml conical tubes
16. 50 ml conical tubes
17. Serological pipets

Protocol:

Tumor Cocktail

1. ____ Prepare Tumor Cocktail in a tissue culture hood in a sterile roller bottle.
2. ____ Add 560ml of RPMI medium 1X, with L-glutamine to the roller bottle.
3. ____ Add 7.5g Dextrose.
4. ____ Add 75ml of Essential amino acids 50X and 140ml of Non-Essential amino acids 100X.
5. ____ Finally add 100ml of sodium pyruvate 100X.
6. ____ Mix ingredients together by swirling.
7. ____ Adjust pH to 7 with 10N NaOH (note 1).
8. ____ After adjusting the pH, add 8.5g of sodium bicarbonate.
9. ____ Then add 100ml of Penicillin/Streptomycin 100X.
10. ____ Bring the volume up to 1L with RPMI.
11. ____ Filter the tumor cocktail through a Nalgene filter unit, 0.2 μ m.
12. ____ Make 47 ml aliquots of tumor cocktail in 50 ml conical tubes.
13. ____ Store at -20°C.

Complete RPMI

14. ____ In a tissue culture hood, add a 47ml aliquot of tumor cocktail to a new bottle of RPMI medium 1X, with L-glutamine.
15. ____ Add 6.5ml of 100X 2-mercaptoethanol (note 2).

16. _____ Finally add 50 ml of FCS (note 3).
17. _____ Mix the media with a 50ml serological pipet by pipetting up and down several times.
18. _____ Store at 4°C (note 4).

Notes:

1. Do not use 10N NaOH from the general lab stock. Make a separate stock and keep it as sterile as possible for future use. Also it is not necessary to use a pH meter, there is a pH indicator in the media. When the media is acidic it is yellow and when it is basic it is red. Adjust the pH so that the color of the media is a bright orangish-red, it will then be at a pH of 7.
2. 100X 2-mercaptoethanol is made by adding 35µl of 2-mercaptoethanol per 100ml RPMI medium 1X, with L-glutamine. This solution is mixed and filtered through 0.2µm filter. Aliquot this solution into 15ml falcon tubes and store at -20°C.
3. FCS is added last because it causes bubbles to form in the media and this should be minimized.
4. This media should be used within 2 months. After 2 months discard and make fresh media.