

SOP: M023

Preparation of Complete RPMI for Growth of THP-1 Cells

Materials and Reagents:

1. ATCC-formulated RPMI-1640 medium, Catalog # 30-2001 stored at 4°C.
2. 2-mercaptoethanol, stock at 55 mM from GIBCO, Cat# 21985-023 stored at 4°C.
3. Fetal Bovine Serum (FBS), not heat inactivated and exosomes free, stored at -20°C.
4. 1 mL pipet tips
5. P1000 pipetman
6. Serological pipet, 50 ml, sterile
7. Electronic pipet-Aid
8. Nalgene filter unit, 0.2 µm
9. Biosafety cabinet

Protocol:

1. _____ Prepare the Biosafety cabinet with all the materials from 11 to 16. (Using aseptic technique. Let on the UV light at least 30 min before starting the process).
2. _____ To prepare the RPMI 1640-complete growth medium add to the base medium:
 - a. 0.05 mM 2-mercaptoethanol (note 1).
 - b. Fetal Bovine Serum (FBS) not heat inactivated, exosomes free (if necessary) at final concentration of 10% (notes 2 & 3).
3. _____ Once the three components are mixed, sterilize the media by filtration through 0.2 µm filter unit (Note 3).
4. _____ Aliquot media into 50 ml Falcon tubes (40 ml per tube).
5. _____ To check for sterility, add the last 10 ml of media to a T25 flask and incubate at 37°C incubator checking every 24 hours up to 72 hours. **Medium should remain red and clear.**
6. _____ Label each 50 ml tube properly (cRPMI, Exosomes Free or not, 10% FBS NOT heat inactivated, date and name initials) and store at 4°C up to one month.

Notes:

1. To 500 ml of RPMI-1640 medium add 454 µL from the stock of 2-mercaptoethanol
2. To 500 ml of media, add 50 ml of FBS (not heat-inactivated, exosomes-free)
3. If exosome free media is needed, the FBS must be cleared of exosomes (SOP: M024). Alternatively, exosome free FBS can be ordered directly from SBI (cat # EXO-FBS-250A-1).
4. To minimize the risk of contamination you must use the vacuum system inside of the biosafety cabinet to filtrate the media.