SOP: M022

Preparation of Complete DMEM for Growth of J774a.1 Cells (Note 1)

Materials and Reagents:

1. Milli-Q water
2. Erlenmeyer, 1 liter
3. Magnetic stir bar
4. Magnetic stir plate
5. DMEM powder (Cellgro cat #50-013-PB)
6. Sodium Bicarbonate
7. HEPES stock 1 M (Hyclone cat #SH30237.01)
8. Sodium Pyruvate stock 100 mM (Hyclone cat #SH30230-01) - 10 ml
9. FBS (Hyclone cat #SH30910.03) – ordered as heat-inactivated or not (notes 2 & 3)
10. Graduated cylinder, 1 liter
11. Serological pipet, 50 ml, sterile
12. Falcon tubes, 50 ml
13. Electronic pipet-Aid
14. Ultracentrifuge
15. Ultracentrifuge tubes
16. Nalgene filter unit, 0.2 μm (note 4)
17. Parafilm
18. Biosafety cabinet

Protocol:

1._____ Prepare the biosafety cabinet with all the materials 11 to 16. (Using aseptic technique. Let on the UV light at least 30 min before starting the process).

2._____ Pour 700 ml of Milli-Q water into a 1 L Erlenmeyer. Add magnetic stir bar and place on magnetic stir plate.

3._____ Add 13.37 g of DMEM powder.

4._____ Add 3.7 g Sodium Bicarbonate.

5._____ Add 10 ml of 1 M HEPES stock.

6._____ Add 10 ml of 100 mM Sodium Pyruvate stock.

7._____ Add Milli-Q water to reach a final volume of 1 L.

8._____ Mix for five minutes covering the Erlenmeyer with parafilm (steps 2 to 9 do not need to be performed in a biosafety cabinet)

9._____ Bring the media to the cabinet. Clean the Erlenmeyer thoroughly with ethanol 70% before put it inside the cabinet.

10._____ Add 100 ml of FBS to the media (FBS 10%) and mix it well.

11._____ Sterilize the media by filtration through 0.2 um filter units (note 4).

12._____ Aliquot media into 50ml Falcon tubes (40 ml per tube).

13._____ 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 remains red and clear.**

14._____ Label each 50 ml tube properly (cDMEM, Exosomes Free or not, 10% FBS heat inactivated, date and name initials) and store a 4°C up to one month.

Notes:

1. Alternatively, DMEM can be ordered from ATCC catalog # 30-2002. Supplement this media with FBS at 10% and follow the steps from 9 to 14 from this SOP. This media contain less sodium bicarbonate concentration (1.5 g/L) this modification is intended for use the media in incubator with CO_2 5%. (The SOP: M021 completely describes the cDMEM preparation from the commercial ATCC DMEM.)
2. If we order the non heat-inactivated we must heat inactivate ourselves before using it in the media. Heat inactivating can be done by heating the serum in a 56°C water bath for 30 minutes. 10% FBS is what the final concentration of FBS should be in the media. So for 1 liter of media you would require 100mL of heat-inactivated FBS.
3. If exosome free media is needed, the FBS must be cleared of exosomes. To do this, ultracentrifuge FBS overnight (12 - 18 hrs) at 4°C at 100,000 xg (28K RPM/SW32 Ti rotor). Exosomes will pellet out and supernatant will be exosome-free FBS. For a detailed protocol see SOP M025.
4. To minimize the risk of contamination you must use the vacuum system inside of the biosafety cabinet to filtrate the media.