

SOP: M024

Preparation of Exosome-Free FBS

Materials and Reagents:

1. Fetal bovine serum (Note 1)
2. Tissue culture/biosafety hood
3. Serological pipet, 50 ml, sterile
4. Electric pipettor
5. Ultracentrifuge, rotor SW28 (swinging bucket)
6. Ultracentrifuge tubes (Beckman Cat # 344058)
7. Tweezers
8. 50 mL Falcon tubes

Protocol:

Day 1

1. _____ Thaw FBS in a 37°C water bath (FBS is stored at -20 upon arrival).
2. _____ In a clean tissue culture hood, add FBS to 6 ultracentrifuge tubes, filling them within 4 – 5 mm from the top (note 2).
3. _____ Weigh tubes and balance within 20 mg of each other (ex: tube 1 & 4 balanced, 2 & 5, 3 & 6).
4. _____ Add balanced tubes to holders to proper slot of rotor. Be sure that they are attached properly and that they appear to hang evenly.
5. _____ Place rotor in ultracentrifuge, close door, set temp to 4°C and start vacuum.
6. _____ Set speed to 28k and time to 0:00 with hold button on (this will allow the run to go until you stop it.) (note 3).
7. _____ Once the second vacuum light is on, hit start and wait for speed to increase.

Day 2

8. _____ Press stop button on ultracentrifuge.
9. _____ Once the speed is at 0, turn off the vacuum, open door and remove rotor.
10. _____ In a tissue culture hood, open each ultracentrifuge tube holder and use tweezers to remove the tube from holder (be sure to only touch the tube and not the FBS with tweezers)
11. _____ Aliquot FBS into 50 ml falcon tubes (note 4).
12. _____ Either add to media immediately or freeze at -20°C for future use.

Notes:

1. Alternatively, exosomes-free FBS can be purchased from SBI (cat # EXO-FBS-250A-1).
2. FBS comes in 500 ml bottles. We can only ultracentrifuge about 200 ml at a time. Freeze the remaining FBS in 50 ml falcon tubes. Be sure to label these tubes properly including ***contains exosomes
3. Ultracentrifuge at least 12 – 18 hrs (longer is fine).
4. Be sure to label the tubes properly. ex: Exosome-free FBS; mm/dd/yy; initials