Supplemental material: Protocol for isolation, stimulation and lysis of PBMC

Materials (listed or equivalent)

BD Vacutainer 9NC (sodium citrate) 6ml (2x), Becton Dickinson (Franklin Lakes, USA), 366575

Falcon 50 mL conical centrifuge tubes, Corning (Corning, USA), 352070

Falcon 15 mL conical centrifuge tubes, Corning (Corning, USA), 352097

Sepmate-50 tubes (or Sepmate-15 for smaller volumes of blood), Stemcell Technologies (Cologne, Germany), 15450

Ficoll, GE Healthcare Life Sciences (Little Chalfont, UK), 17-144-002

PBS ready to use (sterile), VWR International (Radnor, USA), K812-500 ml

Fetal bovine serum (FBS), HyClone (South Logan, USA), SH3007102

Dimethyl sulfoxide (DMSO, sterile), Sigma-Aldrich (Saint Louis, USA), D2438-5X10ML

Hemacytometer, Sigma-Aldrich (Saint Louis, USA), Z359629-1EA

Trypan blue, Sigma-Aldrich (Saint Louis, USA), T8154

RPMI-1640 medium, Gibco, Life Science Technologies (Carlsbad, USA), 21875-034

Phytohaemagglutinin (PHA), Sigma-Aldrich (Saint Louis, USA), L8754-1MG

Interleukin(IL)-2, Sigma-Aldrich (Saint Louis, USA), I7908-10KU

TeloTAGGG Telomerase PCR ELISA plus kit, Roche (Basel, Switzerland), 12013789001

Equipment

Biosafety cabinet

Centrifuge that can cool

Mr. Frosty freezing container with isopropanol

-80 °C freezer (liquid nitrogen tank)

Solutions

Freezing medium: FBS containing 10% DMSO

Culture medium: RPMI-1640 medium containing 10% FBS

PBMC isolation protocol

1. Procedures should be performed in a biosafety cabinet under sterile conditions.
2. Retrieve whole blood.
3. Dilute 1:1 with sterile PBS in 50 ml Falcon tube.
4. Fill Sepmate-50 tube with 15 ml Ficoll.
5. Carefully pipet the diluted blood on the Ficoll layer.
6. Spin down for 10 min at 1200 x g with the brake on.
7. Poor the supernatant within 2 sec in new 50 ml Falcon tube and add PBS until 40 ml.

8. Spin down for 10 min at 300 x g (brake on), remove the supernatant and tap the bottom of tube to loosen the cell pellet.
9. Fill up the tube with 10 ml of PBS.
10. Spin down at 200 x g for 10 min (brake on), remove the supernatant and tap the bottom of tube to loosen the cell pellet.
11. Dissolve the pellet in exactly 1 ml of sterile PBS (mix thoroughly by resuspending).
12. Count cells and calculate the amount of cells/ml using a hemacytometer.
13. Spin down cells at 250 x g for 10 min (brake on), remove the supernatant and tap the bottom of tube to loosen the cell pellet.
14. Dissolve pellet in freezing medium (1 ml of freezing medium / 1 x 10^7 cells).
15. Divide the cells dissolved in the freezing medium over 2 (or more) sterile cryovials and freeze using a Mister Frosty at -80 ºC. Cryovials can be transferred in liquid nitrogen after one day.

PBMC stimulation protocol

1. Procedures should be performed in a biosafety cabinet under sterile conditions.
2. Thaw cells (quickly) in a 37 ˚C water bath and transfer each thawed cell suspension to an appropriately labeled 15 ml Falcon tube and add pre-warmed (37 ˚C) culture medium diluting 10 x the original volume.
3. Centrifuge at 250 x g for 10 minutes at RT, remove the supernatant and tap the bottom of tube to loosen the cell pellet.
4. Resuspend cells in exactly 1 ml of culture medium.
5. Count live cells using a hemacytometer.
6. Dilute the cells in the appropriate amount of culture medium in order to obtain a cell concentration of exactly 1 x 10^6 cells/ml.
7. Pipet 1 ml of cell suspension (1 x 10^6 cells/ml) in the well of a 12-well plate; so each well contains 1 x 10^6 cells.
8. Add 5 µl of Il-2 (10 KU/ml) per ml/well (final concentration = 50 units/ml).
9. Dissolve 1 mg of PHA (Sigma-Aldrich) in 1 ml of PBS (stock concentration = 1 mg/ml).
10. Add 10 µl of PHA (1 mg/ml) per ml/well (final concentration = 10 µg/ml) and incubate 72 hours at 37C° and 5% CO2.

Lysis protocol

1. Procedures should be performed under nuclease free conditions.
2. Take out lysis reagent (Roche TeloTAGGG Telomerase PCR ELISA plus kit) from freezer and let it thaw to 4 ºC. Keep on ice.
3. Label the appropriate amount of sterile (RNase free) 1.5 ml Eppendorf tubes.

4. Remove cells from the wells with a 1000 µl pipet (resuspend a few times to remove aggregates) and place into Eppendorf tubes.
5. Spin down tubes at 300 x g for 10 min at RT, remove the supernatant and tap the bottom of tube to loosen the cell pellet.
6. Dissolve pellet in exactly 1 ml of PBS and carefully resuspend and/or shortly vortex.
7. Count live cells with hemacytometer.
8. Transfer a volume corresponding to exactly 200,000 cells to the corresponding Eppendorf tube:

*required cell volume (µl) = total volume (1000 µl) * 200,000 / total amount of cells*

9. Cool centrifuge and spin down the 200,000 cells for 5 min, 3000 x g at 4 ºC, remove supernatant and keep pellet on ice.
10. Dissolve pellet in 200 µl of cold lysis buffer (Roche TeloTAGGG Telomerase PCR ELISA plus kit).
11. Resuspend pellet and shortly vortex, keep on ice for 30 min.
12. Centrifuge for 20 min, 16,000 x g at 4 ºC.
13. Carefully remove 175 µl of the supernatant, transfer in a sterile labeled (RNase free) 1.5 ml Eppendorf tube and store at -80 ºC.
14. Telomerase activity is measured in the lysates following the instructions of the Roche TeloTAGGG Telomerase PCR ELISA plus kit.