Experimental Protocol

CTCs enrichment using CELLection™ Epithelial Enrich Dynabeads

Total time for the experiment: 90 minutes

Reagents:

CELLection™ Epithelial Enrich Dynabeads, ThermoFisher Scientific #16203
Buffer 1: PBS w/0.1% + BSA pH 7.4.
Buffer 2: PBS (sin Ca2+ ni Mg2+) + w/0.1% BSA + 2mM EDTA
RNAlater® Solution, Ambion #AM7020
50 ml centrifuge tube
15 ml centrifuge tube
1,5 ml eppendorf tubes
25ml, 10ml and 5ml serological disposable pipette
Sterile pipette tips
Plastic pasteur pipettes
Glass pasteur pipettes
Freezer storage boxes for 2ml vials

Instruments and tools:

Centrifuge, capable of ~1250xg with a swing bucket rotor
Magnet (e.g. ThermoFisher Scientific, #12301D)
p200 and p1000 pipette
Rocker shaker
Pipetting aid
-80ºC Temperature Freezer

Prior considerations:

Samples should ideally be processed within 2 hours from the time of extraction.

Steps:

Wash Dynabeads:

- Resuspend the Dynabeads® in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
- Transfer 1 mL of Buffer 1 to a 1,5ml tube.
- Add 100 µl of Dynabeads® to the Buffer1 and resuspend
- Place the tube (open) in a magnet for 1 min and discard the supernatant.
- Remove the tube from the magnet and resuspend the washed Dynabeads® in the same volume of Buffer 1 as the initial volume (100 µl)

Protocol

- Dilute 7.5 mL of whole blood (collected using EDTA tubes) in 15 mL Buffer 2 (1:2) into a 50 ml centrifuge tube.
- Centrifuge at 1250g for 10 min, without brake, at room temperature.
- Discard the plasma fraction/upper layer
- Resuspend in 7.5 mL Buffer 2 at 2°C to 8°C, and transfer to a 15 mL tube.
- Add the 100 µl washed Dynabeads® to the blood.
- Incubate for 30 min at 2°C to 8°C with gentle tilting and rotation.
- Place the open tube in a magnet for 4 min.
- While the tube is still in the magnet, carefully remove and discard the supernatant
- Remove the tube from the magnet and add 1 mL Buffer 1, pipet 2–3 times and transfer to a 1.5 mL tube.
- Place the tube in a magnet for 4 min.
- Repeat the four last steps at least twice to wash the bead-bound cells.
- Resuspend the beads-bound cells in 100 µL of RNA later.
- Store at -80ºC, until RNA extraction and q-RT-PCR characterization

Contact:

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