liquidbio

Procedure

IsoFlux Enriched Sample Beads Removal

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Revision: D

Steps for Live Cells

1. After IsoFlux enrichment, transfer the sample in a 1.5mL microfuge tube (low protein binding preferred).
2. Place the tube on the 1cm diameter magnet (provided with the IsoFlux instrument) for 30 seconds, with the magnet in place, remove and discard the buffer. This is called pull-down method.
3. Re-suspend the sample in 100µL of Binding Buffer
4. Add Papain (Worthington, Product number LK003176) to 1Units/sample. Alternatively FabRICATOR (Sigma, Product number 07298) at 1Unit/sample may be used.
5. Gently flick the tube several times with your finger. Do not agitate or vortex. Incubate at 37°C for 20 minutes.
6. Prepare 5-10mL of RPMI 1640 cell culture medium containing 1% binding buffer, 5% Fetal Bovine Serum. Add 0.5mL of this medium to a new 1.5mL microfuge tube. The released cells in the next step will be added to this tube.
7. Pull down beads, remove and SAVE the supernatant containing the released cells into the microfuge tube prepared in the previous step. Wash the beads once with 50µL of Binding Buffer. Add the wash together with the supernatant.
8. Repeat the digestion once more. Combine the supernatant/released cells.
9. Wash the beads twice with 50µL of Binding Buffer. Add the wash together with the supernatant. Beads may be discarded.
10. Released cells are now ready for analysis or culturing.

Steps for Fixed Cells

1. After IsoFlux enrichment, transfer the sample in a 1.5mL microfuge tube (low protein binding preferred).
2. Fix the sample with Fixative Solution (from IsoFlux enumeration kit) in Binding Buffer at 1:1 dilution for 20 minutes. **Alternatively, other formalin-based fixation is OK.

3. Place the tube on the 1cm diameter magnet (provided with the IsoFlux instrument) for 30 seconds, with the magnet in place, remove and discard the fixation buffer. This is called pull-down method.
4. Wash once with 100µL of Binding Buffer using the pull-down method. Discard the wash.
5. Re-suspend the sample in 100µL of Binding Buffer (This is a potential stopping point. Sample may be stored at 4°C for several days. However, we recommend proceeding to enzyme digestion as soon as possible.)
6. Add Papain (Worthington, Product number LK003176) to 0.5Units/sample (25µL of stock 20Units/mL). Alternatively FabRICATOR (Sigma, Product number 07298) at 1Unit/sample may be used.
7. Gently flick the tube several times. Do not agitate or vortex. Incubate at 37°C for 20 minutes. Pull down beads, remove and SAVE the supernatant containing the released cells into a new microfuge tube.
8. Repeat the digestion once more if desired. Combine the supernatant/release cells. Wash the beads once with 50µL of Binding Buffer. Add the wash together with the supernatant. Beads may be discarded.
9. Add heat-inactivated Fetal Bovine Serum to the released cells to 10% final concentration. Gently re-suspend the sample. Incubate at room temperature for 5 minutes.
10. Centrifuge at 800xg for 5 minutes. Gently remove the supernatant, leaving about 50µL volume containing the cells at the bottom of the tube. Re-suspend in Binding Buffer or PBS if desired. Sample may be stored at 4°C.