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Preparation of PBCs, Lymphocytes, and TRegs for RNA Extraction
(with shipping instructions)

Preparation of the Cell Lysate

1. Following purification or experimental manipulation, store cell samples suspended in working media in polypropylene centrifuge tubes on ice.
2. Assess the number and viability of the cells in an aliquot by Trypan blue exclusion assay. Viability of greater than 90% is preferred for isolation of high quality RNA.
3. Pellet from 5,000 – 20,000,000 cells at 500g for 5 minutes at 4°C in a clinical centrifuge or microfuge.[a] Transfer tubes containing pelleted cells to an ice bucket.
4. Carefully remove the supernatant while avoiding the pellet. For small numbers of cells where the pellet is not readily visible, retain a limited volume of supernatant in the bottom of the tube in order to prevent loss of the pellet.
5. Resuspend cells by gentle pipetting with a wide bore plastic pipette in up to 2.0 ml of ice cold PBS without Ca^{++} and Mg^{++}.
6. Transfer suspended cells to 2 ml RNase-free screw cap tubes (e.g. Light Labs, part # A-8043 tubes and A-8100-R screw caps) pre-equilibrated on ice. [b]
7. Centrifuge at 500g for 5 minutes at 4°C in a microcentrifuge. Transfer tubes back to ice bucket.
8. Carefully remove the PBS supernatant while avoiding the pellet. For small number of cells where the pellet is not readily visible, retain exactly 250 ul of PBS in the bottom of the tube.
9. For visible pellets without residual PBS, pipette 1 ml of Tri-Reagent (Molecular Research Center, part # TR118) in to the tube containing the cell pellet.[c,d] For small cell pellets in 250 ul PBS, pipette 750 ul of Tri-Reagent-LS (Molecular Research Center, part # TS 120) in to the tube. [c,d]
10. Pipette the cell lysate up and down vigorously about 10-20X to thoroughly disperse the cell debris.
11. Freeze tubes on dry ice or store at -80C prior to shipment.

Notes

a, Pricing for gene expression analysis using microarrays or RNA-Seq varies with the amount of available input RNA. For the lowest price, please provide greater than 5,000,000 cells. Note the number and viability of cells on the sample submission checklist.

b, Fisher Scientific, Part # 07-200-210 snap-cap microcentrifuge tubes are also acceptable as are many other commercially available microcentrifuge tubes. Certified RNAse-free tubes, of 1.5 ml – 2.0 ml capacity, rated to 16,000 g are recommended.

c, ORB recommends using a P1000 pipette with 1000 ul RNase-free plastic tip for Trizol addition and subsequent manipulation of lysate.

d, Please read the manual accompanying the Tri-reagent carefully. It is important to immediately pipette the mixture up and down after adding the Tri-Reagent, at the same time the pelleted cells should not be left for lengthy periods on ice. Therefore, it is advisable to minimize the number of PBMC samples to be processed simultaneously.

Shipping

Select a box for shipment that has an external dimension of at least 25 cm in each dimension. The box should have a sturdy cardboard exterior and inner Styrofoam box with wall thickness of at least 2.5 cm. Fill the storage box with 1.5 kg of dry ice. Cool a 133 mm x 133 mm x 48 mm vial storage box in the shipping container. Transfer the frozen sample tubes to the vial storage box. Fill in the free areas of the vial storage box with dry ice and secure the vial storage box top over the samples using wire or string. Fill the Styrofoam shipping container with additional dry ice, and packing peanuts (if necessary) in order to minimize free space in the package. The total dry ice content should be at least 2.5 kg. Use FedEx overnight delivery to ship the package to ORB at the address below. Affix the dry ice sticker to the exterior of the package and record the same dry ice weight on the sticker as was used during set-up of the shipment (may require metric to English unit conversion).

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