

Preparation of Cell Culture Samples for RNA Extraction (with shipping instructions)

Follow the protocols below for adherent and suspension cells respectively. Please read the manual accompanying the Tri-reagent carefully; as these protocols were adapted from that manual.

Adherent Cells

1. Wash one 35 mM plate or a single well of a 6 well plate of cells, per sample, once with ice-cold PBS buffer (containing calcium and magnesium if necessary to prevent detachment).
2. Pipette 1 ml of Tri-Reagent (Molecular Research Center, part # TR118) on to the plate and wash back and forth across the plate. Expand volume accordingly for larger plates.¹
3. Pipette the cell lysate up and down about 10X, washing the lysate across the plate to remove any residual material^a. You may notice increased viscosity due to release of chromosomal DNA from cells at this point; if so continue pipetting up and down until viscosity is reduced.
4. Transfer homogenate to 2 ml RNase-free screw cap tubes (e.g. Light Labs, part # A-8043 tubes and A-8100-R screw caps) at room temperature.²
5. Freeze tubes on dry ice or store at -80C prior to shipment.

Suspension Cells

1. Transfer media containing 2-20 million cells to a polypropylene centrifuge tube (Light Labs, Part # A-8023).
2. Pellet cells at 400g for 5 minutes at 4°C in a clinical centrifuge. Carefully remove media and save pellet.
3. Pipette 1 ml of Tri-Reagent (Molecular Research Center, part # TR118) in to the tube containing cells pellet.¹
4. Pipette the cell lysate up and down about 10X, washing the lysate across the plate to remove any residual material.¹ You may notice increased viscosity due to release of chromosomal DNA from cells at this point; if so continue pipetting up and down until viscosity is reduced.
5. Transfer homogenate to 2 ml RNase-free screw cap tubes (e.g. Light Labs, part # A-8043 tubes and A-8100-R screw caps) at room temperature.²
6. Freeze tubes on dry ice or store at -80C prior to shipment.

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 (2 ml tubes) or 73 mm (5 ml tubes) vial storage box in the shipping container.³ 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).

Attention: David Willoughby
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394 SW 12th Avenue
Deerfield Beach, Florida 33442
Phone: 754-600-5128
Emergency Phone: 561-427-5548

Special Notes

¹, Recommend using a P1000 pipette with 1000 ul RNase-free plastic tip for Trizol addition. A baked glass Pasteur pipette may be used for pipetting lysate up and down if viscosity is an issue; otherwise the P1000 pipette may be used.

², Fisher Scientific, Part # 07-200-210 is also acceptable.

³, The 73 mm deep storage boxes can be ordered from Lab Scientific (part # CBL278).