

File name: RNA Extraction HEK 6well dish ON PLATE.docx

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Summary:

This protocol uses Trizol reagent (Cat #15596) to isolate RNA from a 6-well dish of the following cells:

HEK cells: yield ~50-100ug per well.

Reagents needed:

- Trizol (Life Technologies: Catalog# 15596)
- Chloroform (Fisher: Catalog # C298-500)
- RNase free 1.5 ml tubes with screw cap (MBP Catalog# 3464)
- Isopropanol (Fisher, Catalog# BP2632-4)
- 70% Ethanol (made with ethanol, Fisher, Catalog# BP2818-4 and water, Fisher Cellgro Catalog# MT 46-010-CM)
- Molecular Biology Grade Water (Fisher Cellgro Catalog# MT 46-000-CV)

Protocol:

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- For a 6 well plate: aspirate media and proceed with extraction. Alternatively, plates may be wrapped in foil and frozen at -80degC for extraction at a later date.
- Add 1 ml Trizol to each well of the plate and mix on shaker >5 minutes at room temperature.
- Transfer each sample to a 1.5 ml screw cap tube.
- Add 200 ul CHCl₃ (chloroform), cap tube tightly.
- Vortex vigorously 2x 10 sec, incubate 3 min at room temperature.
- Spin 12k xg 15 min.
- Remove 450 ul of the “top” (~500 ul) aqueous phase (and KEEP!) into a new screw cap tube.

NB: The top aqueous phase contains RNA. Be careful to not disturb the interphase.

- Add 450 ul isopropanol, cap tube and vortex immediately for 10 sec.
- Incubate 10 min at room temperature.
- Spin 12k xg 10 min, remove supernatant and discard in phenol waste.

NB: The white RNA pellet should be visible at the bottom of the tube.

- Add 1 ml 75% EtOH (made with RNase free ethanol and Molecular Biology Grade Water!) and vortex, to wash the RNA pellet.
- Spin 12k xg 5 min.
- Remove and discard the supernatant without disturbing the RNA pellet.

NB: Use a P1000 set to 900ul, followed by a P200 set at 200ul to remove the ~1 ml supernatant.

- Dry the pellet for 5 min in the 65 degC heat block, with the cap open, to remove residual Ethanol.
- Add 100 ul Molecular Biology Grade Water to each RNA pellet. Incubate for 20 min at 65degC in the heat block, with the cap closed tightly. Vortex to mix.