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RNA Extraction using Trizol

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for RNA extraction from cell lines and tissue

Materials

	A	B	C
	Reagent	***Source***	***Identifier***
	TRIzol Reagent	*Life Technologies*	*REF#15596018*
	Linear Acrylamide	*Ambion*	*CAT#AM9520*

RNA Extraction using Trizol

1h 15m 15s

1 1h 15m 15s

From cell lines:

1. Add 1 mL of TRIZOL for RNA isolation.
2. Add 200 µL of chloroform and shake by 00:00:15 vigorously.
3. Leave for 3-10 min at RT to allow for phase separation.
4*. Centrifuge for 00:15:00 at Max speed, 4 °C *
5. Take 3/4 of the upper aqueous phase (very carefully).
6. Mix with the same volume of cold 100% isopropanol as the volume taken from the aqueous phase. (500 µL)
- Optional step:
    ◊ Add 7 µL of acrylamide (if you expect too low amount of RNA from the sample, typically don't need if extracting from 6 well or larger)
7. Gently invert.
8*. Leave for 00:10:00 at RT or longer at -20 °C (If expecting low yield, leave O/N at -20 °C)
9. Centrifuge for 00:15:00 at Max at 4 °C .
10. Discard the supernatant with care.
11. Wash the pellet by 1 mL of 75% (or 70%) Cold EtOH.
12. Vortex (or pipette) the sample briefly to completely wash pellet.
13. Centrifuge for 00:10:00 at max speed at 4 °C .
14. Remove the supernatant.

- Remove supernatant by first dumping into waste container, then draining onto paper towel
- Spin tubes down in microfuge
- Remove residual EtOH from the pellet.

15. Dry the pellet for about 10-20min.

- Dry upside down on kimwipe
- Some people like to dry on the air-intake of the hood

16*. Resuspend the pellet with 15 µL of dH_2O
17. Incubate at 65 °C for 00:05:00 .

*steps different when extracting RNA from **in vivo samples**

4. Spinning for 00:20:00 can help remove "white floaties" from aqueous phase
8. Do not let RNA precipitate for longer than 10 min, usually can start centrifuge step right away

16. Resuspend in 50-60 µL of dH_2O

2 **From tissue samples:**

* Indicate step modification when extracting RNA from **tissue:**

4. Spinning for 00:20:00 can help remove “white floaties” from aqueous phase

8. Do not let RNA precipitate for longer than 10 min, usually can start centrifuge step right away

16. Resuspend in 50-60 µL of dH_2O