TRIZOL RNA PREPARATION

1. Add 1 mL Trizol reagent to cell pellet (from one 100 mm plate of cells).
2. Pipet up and down until viscous.
3. Place on nutator at RT for 5 min.
4. Let sit at RT for 2-3 min.
5. Add 200 μL chloroform.
6. Shake for 10-15 sec.
7. Let sit at RT 2-3 min.
8. Add 200 μl chloroform. Spin 15 min at 4°C, 12 K.
9. Transfer supernatant to new tube (500-550 μl) ***Stay clear of goo.
10. Add 500 μl of isopropanol. Vortex briefly.
11. Let sit at RT for 10 min.
12. Spin for 10 min at 4°C, 12 K (will see white pellet).
13. Aspirate supernatant.
14. Wash pellet with I mL COLD 75% EtOH.
15. Spin 2 min at 4°C, 9 K.
16. Aspirate, but leave about 200 μl *** do not touch pellet. Remove the rest of the liquid with a P200.
17. Invert tubes to dry. *** Do not overdry.
18. Depending on the size of pellet, resuspend in 15-30 μl of RNase-free dH20.

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