Harvesting RNA from 3-D Acinar Cultures

1) Aspirate the media from the wells for harvesting.
2) Add RNA-STAT-60™ (Tel-Test) to each well. For a single well of an 8-well chamber slide, use 250 µl of RNA-STAT-60™. For cultures grown in 24-well plates, use 500 µl of RNA-STAT-60™ per well.
3) Immediately scrape the well to remove the cells and matrigel. For this step, we use a sterile plunger from a 1 ml syringe to scratch the bottom of the well.
4) Resuspend the mixture of RNA-STAT-60™, Matrigel™, and cells several times using a P1000 to break up the Matrigel™.
5) Collect the mixture in an eppendorf tube (approximately 4 wells of a chamber slide per tube).

Following steps are according to manufacturer's instruction:

6) Incubate the mixture 5 minutes at room temperature.
7) Add 200 µl chloroform per 1 ml of RNA-STAT-60™ used to the mixture. (Use 200 µl for 4 wells of an 8-well chamber slide).
8) Shake the tube for 15 seconds in your hand, incubate 2-3 minutes at room temperature.
9) Spin the tubes at maximum speed in a microcentrifuge for 15 minutes at 4°C.
10) Transfer the upper, aqueous phase to a new eppendorf tube.
11) Add 500 µl isopropanol per 1 ml RNA-STAT-60™ used to the aqueous phase.
12) Precipitate the RNA by placing the tubes at 4°C for 30 minutes.
13) Spin the tubes at maximum speed in a microcentrifuge for 10 minutes at 4°C to pellet the RNA.
14) Wash the pellet with 1 ml 75% ethanol.
15) Spin the tubes at 9000x*g* in a microcentrifuge for 5 minutes.
16) Aspirate ethanol and spin once again at 9000x*g* in a microcentrifuge for 2 minutes to remove excess liquid.
17) Dry the pellet at room temperature
18) Rehydrate the RNA pellet for 15 minutes at room temperature with approximately 20 µl DEPC water (for 4 wells of an 8 well chamber slide).
19) Store samples at -80°C.