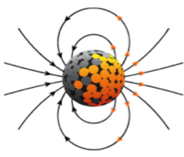

http://magnetics.life
Detroit, MI

Procedure: RNA Isolation for mammalian tissues or xenograft's

Important Note:
1.The lysis buffer could have salt crystals at the bottom of the reagent bottle, in such case warm the reagent to 50º C in a water bath until all the crystal are dissolved.
2. Ethanol is not provided in the kit.
3. Neutralization buffer should be stored at 4º *C.* For long term use aliquot neutralization buffer in 1.5 ml tube(s) and store at 4º *C,* **bring to room temperature before used.**
4. For buffer/lysate/beads mixing steps, make sure you do at least 6 pipette-in or out strokes.
5. Completely remove the cell wash buffer from the cell pellet before starting the lysis step.
Reagent Preparation:
a. Add 22.5 mL of ethanol to the binding buffer bottle.
b. Add 35 mL of ethanol to each Wash buffer bottle.
Procedure:

1. **Lysis:** Add 150 ul of Lysis Buffer to sample containing 1 to 10mg of tissues.
2. Homogenize the tissue by syringe. Homogenize the tissue thoroughly. Make sure no visible clumps are present and the buffer looks clean. **(*Note: If the lysate is too viscous, incubate at room temperature for few minutes and homogenize again*)**
3. **Adding Beads:** Add 50 uL of Life Magnetics (LM) beads onto the cell lysate directly and mix well by pipetting up and down. (*makes an even dark solution*)
4. **Adding neutralization buffer: Bring to room temperature and a**dd 100 uL of Life Magnetics (LM) neutralization buffer onto the cell lysate directly and mix well by pipetting up and down.
5. **Binding:** Add 0.35 mL of binding buffer directly to the lysate-bead solution and mix well by pipetting up and down. (***Note:*** *You should see clumps form in solution if it's working properly)*
6. Place the tubes on the magnetic stand for 90 seconds and aspirate out the supernatant.
7. **Washing:** Add 0.75 mL of wash buffer to the beads, pipette up and down to mix, place the tube back on the magnetic stand for 30 seconds, remove the supernatant. Repeat this step one more time.
8. Add 0.5 mL of wash buffer to the beads, transfer the mixture to a fresh 1.5 mL centrifuge tube and pipette up and down to mix**.**
9. Place the tubes on the magnetic stand for 30 sec and thoroughly remove wash buffer and dry the beads on the bench for 10 mins. (***Note:*** *Ethanol*

promotes binding, allowing all the ethanol to evaporate allows the RNA to come off the beads. Beads will dry much faster if the extraction is performed in the hood)

10. **Elution:** Resuspend the beads into 50 uL to 100 uL elution buffer/TE buffer/RNase-free water. Mix the beads in the elution buffer thoroughly and incubate at 65 C for 5 min to maximize yield. (***Note:*** *resuspend the beads in 50uL of elution buffer for higher concentration of RNA per uL*)
11. After incubation, mix again thoroughly and centrifuge the tube at 10,000g for 2 min and place the tube on the magnetic stand and collect the supernatant. (Alternatively, place the tubes on the magnetic stand for 90 sec and collect the supernatant and place it in a fresh tube)