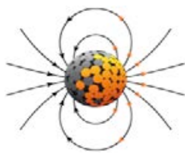

http://magnetics.life
Detroit, MI

Procedure: RNA isolation from saliva

*(**Note:***
1.The lysis buffer could have salt crystals in 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 and Proteinase K are not provided in the kit.
3. For buffer/lysate/beads mixing steps, make sure you do at least 6 pipette-in or out strokes.

Reagent Preparation:

a. Add 4.5mL of water to the *RNA stabilizer bottle and store at 4º C. For longer storage aliquot the solution and freezer them at -20º C.*
b. For every 950µL of lysis buffer add 10µL of RNA stabilizer solution just before the RNA isolation procedure.
c. Add 38mL of ethanol to the binding buffer bottle.
d. Add 35mL of ethanol to each Wash buffer bottle.

1. **Saliva preparation:** For every mL of fresh saliva collection add 50*µL* of RNA stabilizer solution and mix well and place it on ice. Make 1mL aliquots of the saliva samples and centrifuge at 11,000g for 20 min at 4 °C to separate supernatant from the cellular fraction. Keep the cell pellet and the cell-free supernatant in separate tubes.
2. **Lysis:** Add 300µL of Lysis Buffer to the cell pellet sample and mix well. Add 1:1 (do not use more than 300uL/reaction) volume of Lysis Buffer to the supernatant and mix well.
3. **Proteinase K treatment.** Add 450µg of Proteinase K, mix well and incubate for 30 mins at room temperature. Proteinase K treatment is not required for supernatant sample. (Recommended: Proteinase K, recombinant, PCR Grade, Cat# 3115887001 from Sigma, add 25µL/reaction)
4. Add 100µL of Life Magnetics beads, mix thoroughly and incubate at room temperature for 5 mins. (***Note:** Shake the bead bottle thoroughly before using).*
5. **Binding:** Add 700µL of the binding buffer directly to the lysate-bead solution and mix well by pipetting up and down.
6. Place the tubes on the magnetic stand for 2 mins and carefully aspirate out the supernatant.
7. **Washing:** Add 750µL of wash buffer and mix thoroughly by pipetting up and down.
8. Place the tubes on the magnetic stand for 45 sec and thoroughly remove the wash buffer and repeat the steps 7 & 8 one more time.
9. Add 500µL of wash buffer and mix thoroughly by pipetting up and down.

Place the tubes on the magnetic stand for 45 sec and thoroughly remove the wash buffer.

10. Air dry the beads on the bench for 10 mins. (***Note:*** *Allow all the wash buffer to completely evaporate for efficient elution of RNA)*
11. **Elution:** Resuspend the beads into deserved volume (usually 50μL) of elution buffer/TE buffer/water. Mix the beads in the elution buffer thoroughly by flicking the bottom of the tube and incubate at 65 C for 10 min to maximize yield. (***Note:*** *do not mix the beads by pipetting, beads may bind to the pipette tip and reduce the RNA yield. However, pipette could be used for releasing the beads into the elution buffer by scratching off the tube's inner wall, if the beads are firmly bound.*)
12. After incubation, mix again thoroughly by flicking the bottom of the tube and place the tubes on the magnetic stand for 2 mins and collect the supernatant and place it a fresh tube. (Alternatively, centrifuge the tube at 13000g for 2 min and place the tube on the magnetic stand and collect the supernatant.)