**윤상억 교수님 실험 수업 1, 2주차**

**Cells RNA harvest and RNA extraction for qPCR analysis**

Before you begin

1. Add 10 μl β-mercaptoethanol per 1 ml RB Buffer.

2. RB Buffer contains irritant chaotropic salt. Take appropriate laboratory safety precaution, and wear gloves when handling.

1. 6 hours before harvest, the cells prepared in a 60mm dish are treated with IL-1b (20ng/ml).
2. Harvest with 400 μl of RB Buffer and mix with a vortex mixer. Samples must be completely resuspended to achieve maximum dissolution efficiency.
3. **(RNA precipitation)** Add 300μl of ethanol (80%) and mix immediately by using pipette. Do not centrifuge
4. **(RNA binding with spin column)** Transfer the sample to the AccuPrep ® Binding Column-Ⅲ in a 2 ml collection tube, close the lid and centrifuge at ≥ 14,000 rpm for 20sec.
5. Discard the flow-through from the collection tube and reuse the collection tube.
6. **(1st washing)** Add 700 μl of RWA1 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 20 sec. (Note: After centrifugation, carefully remove the AccuPrep ® Binding Column-Ⅲ from the collection tube so that the column does not contact the flow-through. If the rim of column is wet, RNA yield and purity will be less than expected results.)
7. Discard the solution from the collection tube and reuse the collection tube.
8. **(2nd washing)** Add 500 μl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 20 sec.
9. Discard the solution from the collection tube and reuse the collection tube.
10. Add 500 μl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 2 min.
11. Discard the solution from the collection tube and reuse the collection tube.
12. Centrifuge once more at 14,000 rpm for 1 min to completely remove ethanol, and check that there is no droplet clinging to the bottom of the AccuPrep ® Binding Column-Ⅲ tube.
13. **(Elution)** Transfer the AccuPrep ® Binding Column-Ⅲ to a new 1.5 ml tube for elution, add 50μl of ER Buffer onto AccuPrep ® Binding Column-Ⅲ, and wait for at least 1 min at RT (15 - 25℃).
14. Centrifuge at 10,000 rpm for 1 min to elute.