§ Sample Extraction

RNA extraction protocol (Tissue/ cell lines)

Tissue

■ **組織研磨器材準備**

每次研磨完畢之研缽及杵，必須以 3% H_2O_2 浸泡 30 分鐘以上，並以錫箔紙包覆好，置於滅菌釜中滅菌，烘乾隔天使用。

■ **組織研磨**

1. 取碎冰於冰桶中，壓緊碎冰，並在冰上鋪上一層錫箔紙。組織在研磨過程必須在冰上操作。
2. 研缽及杵先以液態氮預冷。
3. 將組織放入研缽中，倒少許的液態氮，快速將組織壓碎，且持續將組織磨至粉狀。(研磨過程，組織必須保持在低溫狀態，需隨時補充液態氮)
4. 至粉狀之後，加入 1 ml TRI Reagnt，小心並快速將 TRI Reagnt 與粉末狀檢體充分混合均勻，此時 TRI Reagnt 會結冰。
5. 待研缽中的檢體慢慢溶解，再將檢體吸入 1.5ml 的離心管中，盡量將研缽中的檢體回收乾淨，(可以用 TRI Reagnt 再次清洗研缽，並收集在另一管 1.5ml 離心管中。)
6. 之後進行 RNA 萃取。(與 Cell lines RNA extraction protocol 相同)

Cell lines

1. Use 1 ml of TRI Reagnt per 10 cm^2 of culture dish area (5~10 x 10^6 cells or 10 cm^2 of culture plate.) or tissue.
2. Trasfer to 1.5ml Eppendorf tube and store the homogenate for 5 minutes at room temperature.
3. Supplement the homogenate with 0.1 ml BCP or 0.2 ml chloroform per 1 ml of Tri-reagent, cover the samples tightly and shake vigorously for 15 seconds. Store the resulting mixture at room temperature for 2-15 minutes and centrifuge at 12,000 g for 15 minutes at 4℃.
4. Transfer the aqueous phase to a new 1.5ml Eppendorf tube. Add 0.5 ml of isopropanol per 1 ml of TRI Reagent and mix well.
5. Store at -80℃ for further purification, 2hrs~over night.

6. Spin RNA sample at maximal speed for 30 min at 4℃.
7. Remove the supernatant and wash the RNA pellet (by vortexing) with 75% ethanol for 2 times.(Add at least 1 ml of 75% ethanol per 1 ml TRI Reagent.)

8. Spin RNA pellet at maximal speed for 5 min at 4℃.
9. Remove the ethanol wash and briefly air-dry the RNA pellet for 3 - 5 min. (**Do not dry RNA by centrifugation under vacuum**.)
10. re-suspend pellet in 100 ul DEPC H_2O.
11. If total RNA yield is < 45 ug, please use RNeasy Micro kit

For QIAGEN RNeasy mini kit purification

(1). 100 ul sample (<100 ug RNA) + 350 ul RLT mix well
(2). add 250ul (96-100％ EtOH). Mix well
(3). Apply sample (~700 ul) to column, spin at max speed rpm for 15 second.
(4). Add 350 ul RW1 into column, spin max speed rpm for 15s
(5). mix 10 ul RNase free DNase I and 70 ul RDD
(6). pipet 80 ul DNase I solution directly on the membrane, place at RT for 15 min
(7). pipet 350 ul RW1 into column and spin 15 sec
(8). change a new collect tube.
(9). Add 500 ul RPE into column, spin for 15 sec
(10). Add another 500 ul RPE, spin at max speed for 2 min
(11). Transfer the column to a new 1.5 ml tube, add 35-50 DEPC H_2O onto membrane, wait for 5-10 min, spin at max sped for 1 min
(12). If RNA yield is < 30 ug, repeat elution step .

For QIAGEN RNeasy Micro kit purification

If total RNA yield is < 45 ug, please use RNeasy Micro kit
(1). 100 ul sample (<100 ug RNA) + 350 ul RLT. Mix well
(2). Add 250ul (96-100％ EtOH). Mix well by pipetting. Do not centrifuge.Continue immediately with step 3.
(3). Apply sample (~700 ul) to column, and centrifuge for 15s at ≧8000*g (≧10,000 rpm).
(4). Pipet 350ul Buffer RW1 into the column, and centrifuge for 15s at ≧8000*g (≧10,000 rpm).
(5). Add 10ul RNase free DNase I to 70 ul Buffer RDD.
(6). Pipet 80 ul DNase I mix solution directly onto the membrane, and place at room temperature (RT) for 15 min.
(7). Pipet 350 ul RW1 into column and spin 15s at ≧8000*g
(8). Transfer the new 2ml collection tube.
(9). Pipet 500ul RPE onto the column. And centrifuge for 15s at ≧8000*g (≧10,000 rpm).

(10). Add 500ul 80% ethanol to the column, and centrifuge for 2 min at ≧8000*g (≧10,000 rpm) to dry the membrane.
(11). Transfer the a new 2ml collection tube. Open the cap and centrifuge at full speed for 5 min.
(12). To elute, transfer the new 1.5ml collection tube. Pipet 14 ul DEPC H_2O onto membrane, wait for 5-10 min, and centrifuge for 1 min at maximum speed to elute.

Thing to do before starting

1.Prepare 80% ethanol
2.Prepare 96~100% ethanol