BECKMAN COULTER
Life Sciences

Simultaneous DNA&RNA Extraction from Tissue without Splitting Lysate

Applicable for scientists working on precious tissue sample who want to extract DNA and RNA.

Please reference the content FormaPure XL Total (Part number: C35991 or C35992) and RNAdvance Tissue (Part number: A32645, A32649 or A32646) for product information.

Purpose

With the advance of Next Generation Sequencing (NGS), researchers now can access both genomic and transcriptomic data from a single sample. The ability to isolate high-quality DNA and RNA from the same biological sample is becoming increasingly important, especially when the sample yields small amount of nucleic acid. We developed a protocol using Beckman Coulter reagents to isolate both DNA and RNA from the same fresh/frozen sample. The method utilizes size selection to separate DNA and RNA.

Material needed

Material	Part Number	Supplier
RNAdvance Tissue Lysis Buffer	A32645, A32646 or A32649	Beckman Coulter
RNAdvance Tissue PK	A32645, A32646 or A32649	Beckman Coulter
FormaPure Total Bind (BBA)	B85603	Beckman Coulter
FormaPure Total Rebind (RBA)	C16684	Beckman Coulter
FormaPure Total Wash (WBA)	B85625	Beckman Coulter
FormaPure Total RNase A	B85620	Beckman Coulter
100% Ethanol (Molecular Grade)	AB-00138	American Bio
DNase I (RNase-free)	AM2222 or AM2224	ThermoFisher Scientific Ambion
Nuclease-free water (Molecular Grade)	W4502	Sigma-Aldrich
7 Bar Magnet for 96–Well Plate	771MWZM-1ALT	V&P Scientific
1.2 mL 96-well plate	AB1127	ThermoFisher Scientific
37 degree Heat Block or Water Bath | N/A	N/A	N/A

Protocol

1. **Homogenization / Lysis**
    a. Transfer **10 mg tissue** in a 1.5 mL centrifuge tube or 1.2 mL 96 well plate
    b. Add **380 µL RNAdvance Lysis buffer** and **20 µL Proteinase K** to the plate
    c. **Homogenize** the tissue: please use either tissuelyser (steel ball) or hand homogenizer
    Adjust the speed and time to make sure proper tissue homogenization
    After homogenization, if tissuelyser is used, remove the steel ball
    d. **Incubate** the tissue at **37°C** for **25 minutes**
    e. **Centrifuge** the sample at **12000 rpm** for **1 minute**
    f. Transfer the supernatant to a new 96-well plate. Discard tube or plate of undigested tissue

2. **Bind**
    a. Vortex to fully resuspend the **FormaPure Bind Buffer (BBA)**
    b. Add **150 µL** of **BBA** to the plate in 1f
    c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    d. **Incubate** at **room temperature** for **5 minutes**
    e. Place the sample on a **magnet** for **10 minutes** (or until the beads are settle)
    f. Fully transfer the **supernatant (550 µL)** from the plate to a new 1.2 mL 96 well plate. The supernatant will be processed for **RNA extraction** as follows:

3. **RNA extraction**
    a. Bind
        i. Add **550 µL** of **BBA** to the supernatant from step 2f
        ii. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
        iii. **Incubate** at **room temperature for 5 minutes**
        iv. Place on magnet for **10 minutes** (or until the beads are settled)
        v. Fully remove supernatant from the plate and discard supernatant
        vi. Remove plate from magnet
    b. Wash
        i. Add **800 µL** of **80% ethanol** to the plate
        ii. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
        iii. Place on magnet for **3 minutes** (or until the beads are settled)
        iv. Fully remove supernatant from the plate and discard supernatant
        v. Remove plate from magnet
    c. DNase treatment
        i. Add **100 µL** of **DNase solution.**
           DNase solution is prepared as instructed in the FormaPure XL Total protocol
            - **80 µL** of **Nuclease free water**
            - **10 µL** of **DNase**
            - **10 µL** of **DNase Buffer**
        ii. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
        iii. **Incubate** at **37°C** for **20 minutes**
    d. Rebind
        i. Add **150 µL** of **Rebind Buffer (RBA)**
        ii. **Mix** by pipetting up and down 10 times (or until thoroughly mixed)
        iii. **Incubate** at **room temperature** for **5 minutes**
        iv. Place on magnet for **10 minutes** (or until the beads are settled)
        v. Fully remove supernatant from the plate and discard supernatant
        vi. Remove plate from magnet

e. Wash
    i. Add **800 µL** of **80% ethanol** to the plate
    ii. **Mix** by pipetting up and down 10 times (or until thoroughly mixed)
    iii. Place on magnet for **3 minutes** (or until the beads are settled)
    iv. Fully remove supernatant from the plate and discard supernatant
    v. Remove plate from magnet

f. Elute
    i. Add **40 µL** of **nuclease free water** to the plate
    ii. **Incubate** at **60°C** for **2 minutes** while shaking at **300 rpm**
    iii. Place on magnet for **5 minutes**
    iv. Remove and **save** the supernatant without disrupting the beads

4. **DNA extraction**

The beads part will be processed for DNA extraction as follows:

a. RNase treatment
    i. Remove plate from magnet
    ii. Add **225 µL** of **WBA** and **5 µL** of **RNase** to beads
    iii. **Mix** by pipetting up and down 10 times (or until thoroughly mixed)
    iv. **Incubate** at **room temperature** for **5 minutes**
    v. Place on magnet for **10 minutes** (or until the beads are settled)
    vi. Fully remove supernatant from the plate and discard supernatant
    vii. Remove plate from magnet.

b. Wash
    i. Add **800 µL** of **70% ethanol** to the plate
    ii. **Mix** by pipetting up and down 10 times (or until thoroughly mixed)
    iii. Place on magnet for **10 minutes** (or until the beads are settled)
    iv. Fully remove supernatant from the plate and discard supernatant
    v. Remove plate from magnet

c. Elute
    i. Add **40 µL** of **nuclease free water** to the plate
    ii. **Mix** by pipetting up and down 10 times (or until thoroughly mixed)
    iii. **Incubate** at **60°C** for **2 minutes** while shaking at **300 rpm**
    iv. Place on magnet for **5 minutes**
    v. Remove and **save** the supernatant without disrupting the beads

Results

Frozen mouse liver tissue was used for this protocol demonstration. DNA was measured by Quant-iT™ PicoGreen® dsDNA Assay (Thermo Fisher Scientific) to assess the yield (Figure 1). From this experiment, we got 32 µg DNA per 10 mg mouse liver tissue.

RNA was tested for Quant-iT™ RiboGreen® RNA Reagent. Per 10 mg mouse liver tissue, about 66 µg RNA was extracted (Figure 1).

High integrity of the nucleic acid was obtained by using this protocol as measured by Agilent TapeScreen assay: DIN 9.2 and RIN 7.4 (Figure 2).

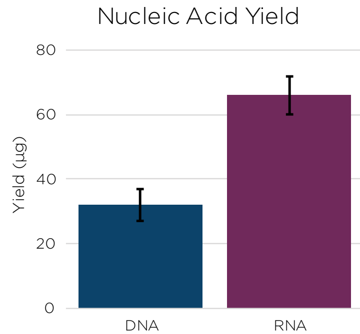

Figure 1. DNA and RNA yield were tested. DNA and RNA were measured by Quant-iT™ PicoGreen® dsDNA Assay and Quant-iT™ RiboGreen® RNA Reagent.

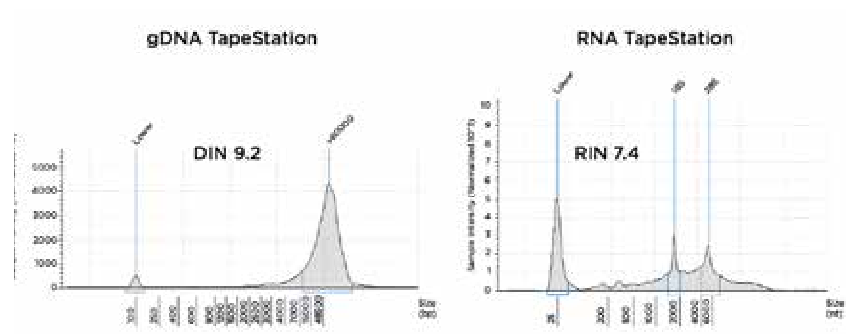

Figure 2. High integrity of DNA and RNA were obtained from the workflow.

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