BECKMAN COULTER
Life Sciences

Simultaneous DNA & RNA extraction from cultured cells without splitting lysate

This method is applicable for scientist who want to extract both DNA and RNA from the same sample of cultured cells.

Please reference the current FromaPure Total IFU (Part number: C16675 or C16676) and RNAdvance Cell V2 IFU (Part number: A47942 or A47943) for product information.

Purpose

Advances in Next Generation Sequencing (NGS) increases researchers need to 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 amounts of total nucleic acids. We developed a protocol using Beckman Coulter reagents to isolate both DNA and RNA from the same cultured cell sample. The method utilizes a proprietary buffer to selectively bind RNA and DNA.

Material Used

Material	Part Number	Supplier
RNAdvance Cell v2 Lysis Buffer	A47942, A47943	Beckman Coulter
RNAdvance Cell v2 Proteinase K	A47942, A47943	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	B85620	Beckman Coulter
Microcentrifuge tubes 1.5 mL	357448	Beckman Coulter
Tube Magnet (1.5, 1.7, and 2 ml)	A29182	Beckman Coulter
Ethanol	AB–00138	American Bioanalytical
DNase I (RNase–free)	AM2222 or AM2224	ThermoFisher Scientific Ambion
Nuclease–free water (Molecular Grade)	AM9932	ThermoFisher Scientific
RNase A	B85620	Beckman Coulter
Phosphate Buffered Saline Solution, 10x Concentrate	AB11072-01000	AmericanBio
37 degree Heat Block or Water Bath	N/A	N/A
60 degree Heat Block or Water Bath	N/A	N/A
Microcentrifuge	N/A	N/A

Protocol

1. **Lysis**
    a. Dilute cells to have **1000 T cells** in **10 µL 1XPBS** per tube.
    b. Add **10 µL** of cells to a **1.5 mL microcentrifuge** tube
    c. Add **100 µL** of **RNAdvance Cell v2 lysis buffer** to the sample
    d. Add **5 µL** of **Proteinase K** to the sample
    e. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    f. **Incubate** the sample for **30 minutes** at **room temperature**
        i. Alternatively **incubate** the sample for **15 minutes** at **37°C**

2. **Bind**
    a. Vortex the bottle of **BBA** to fully resuspend the beads
    b. Prepare **50 % BBA**:
        i. **55 µL** of **BBA**
        ii. **55 µL** of **nuclease–free water**
    c. Add **110 µL** of **50% BBA** to the sample
    d. **Mix** up and down with pipette 10 times, or until thoroughly mixed
    e. **Incubate** the sample at for **5 minutes** at **room temperature**
    f. Place the sample on a magnet for **10 minutes** (or until the supernatant is clear)
    g. Remove and **transfer** the **supernatant** from the tube to a new 1.5 mL microcentrifuge tube
        i The supernatant will be processed for **RNA extraction** as follows
        ii. Save 1.5 mL tube for step 4. **DNA extraction**

3. **RNA extraction**
    a. **Bind**
        i. Add **200 µL** of **BBA** to the **supernatant** from step 2.g.
        ii. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
        iii. **Incubate** for **5 minutes** at **room temperature**
        iv. Place the sample on a magnet for **10 minutes** (or until supernatant is clear)
        v. Remove and discard the supernatant without disrupting the beads
        vi. Remove the sample from the magnet

    b. **Wash**
        i. Add **375 µL** of **80% ethanol** to the sample
        ii. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
        iii. Place the sample on a magnet for **3 minutes** (or until supernatant is clear)
        iv. Remove and discard the supernatant without disrupting the beads
        v. Remove the sample from the magnet

c. **DNase Treatment**
   i. Add **100 µL** of **DNase solution** to the sample
      a. DNase solution is prepared as instructed in the FormaPure Total IFU
         i. **80 µL** of **nuclease free water**
         ii. **10 µL** of **DNase I**
         iii. **10 µL** of **10x DNase Buffer**
   ii. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   iii. **Incubate** for **20 minutes** at **37°C**

d. **Rebind**
   i. Add **250 µL** of **RBA** to the sample.
   ii. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   iii. **Incubate** for **5 minutes** at **room temperature**
   iv. Place the sample on a magnet for **10 minutes** (or until supernatant is clear)
   v. Remove and discard the supernatant without disrupting the beads
   vi. Remove the sample from the magnet

e. **Wash**
   i. Add **375 µL** of **80% ethanol** to the sample
   ii. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   iii. Place the sample on a magnet for **3 minutes** (or until supernatant is clear)
   iv. Remove and discard the supernatant without disrupting the beads
   v. Remove the sample from the magnet

f. Elute
   i. Add **40 µL** of **nuclease free water** to thesample
   ii. Incubate for **2 minute** at **60°C**
   iii. Place the sample on a magnet for **5 minutes** (or until supernatant is clear)
   iv. Remove and **save** the supernatant without disrupting the beads

4. DNA extraction
The beads from Step 2.g will be processed for DNA extraction as follows:

a. **RNase treatment**
   i. Remove the sample from the magnet.
   ii. Add **300µL** of **WBA** to the sample
   iii. Add **2.5µL** of **RNase** to the sample
   iv. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   v. Incubate for **5 minutes** at **room temperature**
   vi. Place the sample on a **magnet** for **10 minutes** (or until supernatant is clear)
   vii. Remove and discard the supernatant without disrupting the beads
   viii. Remove the sample from the magnet

b. **Wash**
   i. Add **375 µL** of **70% ethanol** to the sample
   ii. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   iii. Place the sample on a **magnet** for **10 minutes** (or the supernatant is clear)
   iv. Remove and discard the supernatant without disrupting the beads
   v. Remove the sample from the magnet

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

Results

Human T lymphocyte cell line (Jurkat) was used for protocol demonstration. We extracted DNA and RNA from increasing cell amounts: 10000, 50000, 100000 and 500000. DNA concentration was measured by Quant-iT™ PicoGreen® dsDNA Assay (Thermo Fisher Scientific) (Figure 1). RNA concentration was measured by Quant-iT™ RiboGreen® RNA Reagent (Figure 1). The average yield was 171 ng of DNA and 94 ng RNA from 10000 cells. Both DNA and RNA yield increased in a cell number–dependent manner.

High integrity nucleic acid was obtained by using this protocol as measured by Agilent TapeScreen assay: DIN above 9.0 and RIN above 9.2 (Figure 2). The 10000 cell number group showed a lower nucleic acid integrity (DIN 6.7 and RIN 8.0), we believe that nucleic acid yield from 10000 cells were below the quantitation range of the ScreenTape assay, thus it affected the reading.

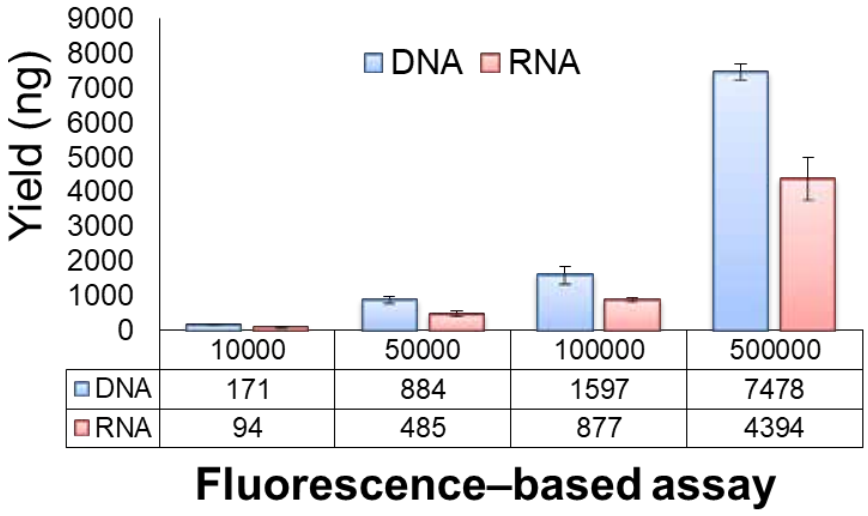

Figure 1. DNA and RNA yield form single cell lysate sample using BEC extraction method. The yield was measured by Quant-iT PicoGreen dsDNA Assay (Thermo Fisher Scientific) or Quant-iT RiboGreen RNA Assay (Thermo Fisher Scientific).

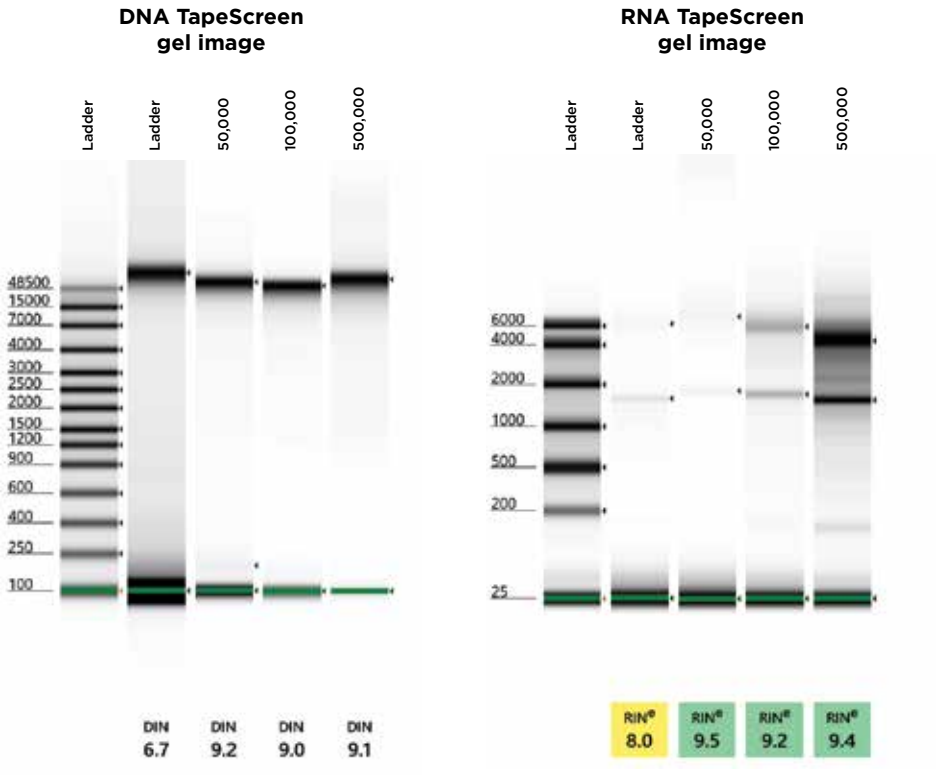

Figure 2. DNA and RNA quality form single cell lysate sample using BEC extraction method. Agilent DNA or RNA TapeScreen assay was used to access the nucleic acid integrity.

AAG-5118SP04.19