User's Guide

# *AccuPrep*®
# Universal RNA Extraction Kit

REF K-3140, K-3141

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# *AccuPrep*® Universal RNA Extraction Kit

# User's Guide

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**Please read all the information in booklet before using the unit**

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## Safety Warnings and Precautions

For research use only

Not recommended for disease diagnose in humans or animals.

Wear gloves when you are handling irritant or harmful reagents.

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# I. Description

*AccuPrep*® RNA Extraction Kit can quickly and conveniently extract RNA from cultured cell, plant and animal tissue. In the presence of chaotropic salt, RNA is bound to glass fibers fixed in a column. Proteins and other contaminants are removed through washing steps, and the RNA is isolated and eluted in the final elution step. The process does not require the use of organic solvents or ethanol precipitation steps, and is thus ideal for the safe and convenient extraction.

Protocol is suitable for use with cultured cell, plant and animal tissue. We recommended DNase treatment for only RNA quantitation.

## Advantages

1. RNA can be prepared more promptly and conveniently.
2. Other cellular components besides nucleic acids, especially proteins, nucleases, and other contaminants, are completely eliminated, resulting in improved efficiency and reproducibility of RT reaction.
3. Damage and low yield of RNA are minimized because the all steps proceed without organic solvent and ethanol precipitation process.
4. The extracted RNA can be used in a variety of applications.

## II. Kit components

This kit provides for 100 preparations and will maintain performance for at least two years under standard storage conditions.

### *AccuPrep*® Universal RNA Extraction Kit (K-3140, K-3141)

| Reagents | K-3141 (50 rxn) | K-3140 (100 rxn) |
|---|---|---|
| RB Buffer | 28 mL | 56 mL |
| RB Buffer is stable for 2 years when stored at room temperature. | | |
| RWA1 Buffer | 40 mL | 80 mL |
| RWA1 Buffer is stable for 2 years when stored closed at room temperature. | | |
| RWA2 Buffer | 70 mL | 70 mL (x 2) |
| RWA2 Buffer is stable for 2 years when stored closed at room temperature. | | |
| ER Buffer | 10 mL | 20 mL |
| Store at room temperature. | | |

| Columns and tubes | 50 rxn | 100 rxn |
|---|---|---|
| *AccuPrep*® Binding Column-III | 50 ea | 100 ea |
| Collection tube (for filtration) | 50 ea | 50 ea (x 2) |
| 1.5 ml tubes for elution | 50 ea | 50 ea (x 2) |

### Additional required materials

- Absolute ethanol (96 – 100%)
- 1.5 ml tube (for preparation of lysis)
- Standard table-top microcentrifuge capable of a 13,000 x *g* centrifugal force (with rotor for 2 ml tubes)
- Incubator or thermal block
- Vortex mixer
- Sterilized filter pipette tip

## III. Experimental protocol

### 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.

## A. RNA Extraction from Cultured Cell

1) (Harvest cell) Cells grown in suspension:
   Count the cell number and centrifuge given number of cells ($10^4$ – $10^6$) at 300 x *g* for 5 min. Discard supernatant carefully without disturbing the pellet and go to lysis & homogenization (go to step 3).

2) Cells grown in a monolayer: There are 2 different ways to collect cells grown in a monolayer.
   a. Direct cell lysis on the culture dish:
      Completely remove cell culture medium and go to lysis & homogenization (go to step 3). (Remaining medium may inhibit the RNA extraction)
   b. Harvesting cells with trypsin:
      Remove cell culture medium and wash the monolayer with DPBS. Add 0.1% – 0.25% trypsin to the washed cell monolayer. When the cells are detached, add cell culture medium to inactivate the trypsin. Transfer the cells into a RNase-free tube and centrifuge at 300 x *g* for 5 min. Discard supernatant carefully and go to lysis & homogenization (go to step 3).

3) (Lysis & homogenization) Add 400 μl of RB Buffer to the cell pellet and mix by vortex mixer.
   Make sure that you must completely resuspend the sample to achieve maximum lysis efficiency.
   (Note: Incomplete homogenization leads to significantly reduced RNA yields.)

4) (RNA precipitation) Add 300 μl of ethanol (80%) and mix immediately by using pipette. Do not centrifuge.
   (Note: When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.)

5) (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 20 sec.
   If the sample volume exceeds 700 μl, centrifuge successive aliquots in the same binding column. Discard the flow-through after each centrifugation.

6) Discard the flow-through from the collection tube and reuse the collection tube.

7) (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.)

8) Discard the solution from the collection tube and reuse the collection tube.

9) (2nd washing) Add 500 μl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 20 sec.

10) Discard the solution from the collection tube and reuse the collection tube.

11) Add 500 μl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 2 min.
The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.

12) Discard the solution from the collection tube and reuse the collection tube.

13) 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.

14) (Elution) Transfer the *AccuPrep*® Binding Column-III to a new 1.5 ml tube for elution, add 50 – 200 μl of ER Buffer onto *AccuPrep*® Binding Column-III, and wait for at least 1 min at RT (15 – 25℃).

15) Centrifuge at 10,000 rpm for 1 min to elute.
If the expected RNA yield is > 30 μg, repeat step 14 &15 using another 50 – 100 μl ER Buffer (if high RNA concentration is required). Reuse the collection tube from step 15.
(If using the eluate step, the RNA yield will be less than that obtained using a second volume of ER Buffer, but the final RNA concentration will be higher.)

## B. RNA Extraction from Plant Tissue

1) (Sample preparation) Grind sample (up to 100 mg) under liquid nitrogen to a fine powder using a mortar and pestle. Transfer the tissue powder and liquid nitrogen to an appropriately sized tube, and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. Continue immediately with step 2.

2) (Lysis & homogenization) Add 500 μl of RB Buffer to a maximum of 100 mg tissue powder and vortex vigorously.

3) Incubate at 60°C for 1 – 3 min. Centrifuge at full speed for 2 min and transfer the supernatant to a new microcentrifuge tube.
A short 1 – 3 min incubation at 60°C may help to disrupt the tissue.
Optional: Centrifugation through the *AccuPrep*® pre-column removes debris effectively.

4) (RNA precipitation) Add 0.5 volume of ethanol (96 – 100%) and mix immediately by using pipette. Do not centrifuge.

5) Go to step 5 of "RNA Extraction from Cultured Cell" in page 3 and follow the instructions accordingly.

## C. RNA Extraction from Animal Tissue

1) (Lysis & homogenization) Homogenize the sample (20 – 30 mg) with a homogenizer, place them in a clean 1.5 ml tube, and add 500 μl of RB Buffer.

2) Centrifuge the lysate for 3 min at full speed. Carefully transfer the supernatant to a new microcentrifuge tube by pipetting. Use only this supernatant (lysate) in subsequent steps.

3) (RNA precipitation) Add 200 μl of ethanol (96–100%) and mix immediately by using pipette. Do not centrifuge.
Optional: Centrifugation through the *AccuPrep*® pre-column removes debris effectively.

4) Go to step 5 of "RNA Extraction from Cultured Cell" in page 3 and follow the instructions

accordingly.

## D. RNA Clean-up

1) Adjust the sample to a volume of 100 µl with RNase-free water. Add 400 µl RB Buffer, and mix well.
2) Add 300 µl of ethanol (80%) to the diluted RNA, and mix immediately by using pipette. Do not centrifuge.
3) 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 20 sec.
4) Discard the flow-through from the collection tube and reuse the collection tube.
5) Add 500 µl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 20 sec.
6) Discard the solution from the collection tube and reuse the collection tube.
7) Add 500 µl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 2 min.
   The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.
8) Discard the solution from the collection tube and use a new collection tube.
9) 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.
10) Transfer the *AccuPrep*® Binding Column-Ⅲ to a new 1.5 ml tube for elution, add 50 – 200 µl of ER Buffer onto *AccuPrep*® Binding Column-Ⅲ, and wait for at least 1 min at RT (15 – 25℃).
11) Centrifuge at 10,000 rpm for 1 min to elute.

## Summary of reagent volumes required in each step of RNA Extraction

### RNA Extraction from Cultured Cell

| Step | Buffer | Volume |
|---|---|---|
| Cell Lysis & Binding | RB Buffer | 400 µl |
| RNA Precipitation | 80% Ethanol | 300 µl |
| 1st Washing | RWA1 Buffer | 700 µl |
| 2nd Washing (x 2) | RWA2 Buffer | 500 µl |
| Elution | ER Buffer | 50 – 200 µl |

### RNA Extraction from Plant Tissue

| Step | Buffer | Volume |
|---|---|---|
| Cell Lysis & Binding | RB Buffer | 500 µl |
| RNA Precipitation | Absolute Ethanol | 0.5 volume |
| 1st Washing | RWA1 Buffer | 700 µl |
| 2nd Washing (x 2) | RWA2 Buffer | 500 µl |
| Elution | ER Buffer | 50 – 200 µl |

### RNA Extraction from Animal Tissue

| Step | Buffer | Volume |
|---|---|---|
| Cell Lysis & Binding | RB Buffer | 500 µl |
| RNA Precipitation | Absolute ethanol | 200 µl |
| 1st Washing | RWA1 Buffer | 700 µl |
| 2nd Washing (x 2) | RWA2 Buffer | 500 µl |
| Elution | ER Buffer | 50 – 200 µl |

# IV. Appendix

## A. Troubleshooting guide

If you have problems during RNA extraction, please use the Troubleshooting Guide. This troubleshooting guide will help you to solve problem that may arise during RNA extraction. For other technical assistance or more information, please contact our technical assistance team.

| | Comments and suggestions |
|---|---|
| Low yield of RNA | Buffers or other reagents may have been exposed to external factors that may have reduced its quality. Please make sure that reagents are stored at room temperature at all times upon arrival and that all reagent bottles are closed tightly, in order to preserve pH and stability, and to avoid contamination. |
| | Excess amount of starting sample was used to extract DNA. Appropriate amount of starting sample should be used for efficient extraction of RNA. |
| | Elution may have been incomplete. Please extend incubation time up to 3 minutes at elution step to improve the RNA. |
| | Insufficient shaking or vortexing during lysis step may lead to low RNA yield than expected. Shake or mix with a vortex mixer sufficiently during incubation step. |
| | Cell culture medium may have been incomplete. The best approach is to remove the medium as much as possible. Any leftover in the medium can lead to the inhibition of RNA extraction. |
| Low $A_{260/280}$ ratio | Spin column may have been washed insufficiently. You must properly wash the spin column in the washing step. Remaining ethanol can decrease the purity of RNA. |
| Degraded RNA | RNase contamination can be degraded RNA. Use a heat gun or a blow dryer in a clean bench to prevent the contamination of RNase in the air. Use RNase-free pipette tips and change the gloves frequently. |
| | Cultured cell samples that have been stored at -80℃ or lysis the samples with RB Buffer and then store at -80℃. |
| | Frequent freezing and thawing may result in lower RNA yield than expected. Avoid repeated freezing and thawing. |
| Flotation of extracted RNA when loaded on an agarose gel | Floating of RNA on an agarose gel is caused by the remaining ethanol in the eluted RNA. Ensure that the ethanol removing step in the protocol is properly performed. Remaining ethanol may also interrupt the enzymatic reaction. |

## B. Long term storage of RNA in formamide

1) Dissolve RNA pellet in deionized formamide.
2) To precipitate RNA from formamide, add NaCl to final concentration of 0.2 M followed by 4 volumes of ethanol.
3) Incubate at room temperature for 10 min.
4) Centrifuge at 12,000 rpm for 5 min at room temperature.

## C. Measuring absorbance of RNA samples

The $A_{260}/A_{280}$ ratio is a commonly used criterion for nucleic acid purity. Values for pure RNA are usually > 1.8. However, the absorbance of nucleic acids at these wavelengths is dependent upon the ionic strength and pH of the medium. The change in the $A_{260}/A_{280}$ ratio is primarily due to the decrease in the absorbance at 280 nm when the ionic strength or pH is increased. We recommend that RNA be diluted with TE buffer for spectrophotometric assays.

1) Measure the total RNA sample volume.
2) Transfer 1 μl of your total RNA sample to a 1.5 ml tube.
3) Add 999 μl of TE (pH 8.0) buffer to the 1.5 ml tube and mix by pipetting.
4) Measure $A_{260}$ and $A_{280}$ using TE (pH 8.0) buffer as a reference blank.
5) Calculate RNA yield as follows:

   1$A_{260}$ unit of RNA = 40 μg/μl

   Total $A_{260}$ = ($A_{260}$ of dilute sample) x (dilution factor)

   Concentration (μg/ml) = (total $A_{260}$) x (40 μg/μl)

   Yield(μg) = (total sample volume) x (concentration)
6) Calculate the $A_{260}/A_{280}$ ratio. Pure RNA exhibits a ratio between 1.9 – 2.0.

**Example:** The volume of RNA sample was 0.5 ml. One 1 μl sample aliquot was diluted to 1.0 ml in TE (pH 8.0) buffer, and the spectrophotometric readings were taken: $A_{260}$ = 0.231, $A_{280}$ = 0.115

**Caculations:**

Total $A_{260}$ = (0.131) x (1000) = 131

Concentration = (131) x (40) = 5,240 μg/ml

RNA yield = (0.5 ml) x (5,240 μg/ml) = 2,620 μg

Purity = 0.131 / 0.065 = 2.01

## V. References


1. N. J. Coombs. *et al.* (1999) *Nucleic Acids Res.,* Vol **27**, No.16
2. C. Reno. *et al.* (1997) *Biotechniques* , Vol **22**, No. 6
3. Michael J.Bonham. *et al.* (1996) *Biotechniques,* Vol **20**, No. 5


## VI. Explanation of symbol

| Symbol | Meaning | Symbol | Meaning |
|---|---|---|---|
| | Catalog Number | | Contains sufficient for (n) tests |
| | USE BY | | Temperature Limitation |
| | Batch code | | Caution, consult accompanying documents |
| | Manufacturer | | Caution, Potential Biohazard |
| | DO NOT REUSE | | Consult Instruction For Use |

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