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(V1/2023-06-14)
One page protocol

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

Ⅰ Before You Begin

1) Add 10 µl of β-mercaptoethanol per 1 ml RB Buffer.
2) Prepare additional ethanol (80% and 100%) that is not included.
3) Add correct amount of absolute ethanol to RWB1 Buffer and RWB2 Buffer, respectively (see bottle label).

Ⅱ Cultured Cell Collection

1) Suspension cell culture:
   Harvest cultured cells (10^4-10^6 cells) by centrifugation at 300 x *g* for 5 min to pellet cells. Discard supernatant carefully without disturbing a cell pellet and go to step Ⅲ.
2) Monolayer cell culture: There are 2 different ways to collect cells grown in monolayer culture.
   a. Direct cell harvesting on the culture dishes: Completely remove cell culture medium and go to step Ⅲ.
   b. Cell harvesting with trypsin: Remove cell culture medium and wash the cell monolayer with DPBS. Add 0.1-0.25% typsin to the washed cell monolayer to detach the cells. When the cells are detached, add cell culture medium to inactivate typsin. Transfer the cells into a RNase-free tube and centrifuge at 300 x *g* for 5 min. Discard supernatant carefully and go to step Ⅲ.

Ⅲ RNA Extraction from Cultured Cell

1) Add **400 µl** of **RB Buffer** to the cell pellet and mix by vortex mixer.
2) Add **300 µl** of **80% ethanol** and mix immediately by using pipette.
3) Transfer the sample to a Binding column fitted in a 2 ml collection tube.
4) Close the lid and centrifuge at **≥14,000 rpm** for **20 sec**.
5) Discard the flow-through from the collection tube and reuse the collection tube.
6) Add **700 µl** of **RWB1 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm** for **20 sec**.
7) Discard the solution from the collection tube and reuse the collection tube.
8) Add **500 µl** of **RWB2 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 **RWB2 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.

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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 binding column tube.
13) Transfer the Binding column tube to a new 1.5 ml tube (supplied with a kit) for elution, add **50-200 µl** of **ER Buffer** onto Binding column tube, and wait for at least 1 min at RT (15-25℃).
14) Centrifuge at **10,000 rpm** for **1 min** to elute.

Ⅳ RNA Extraction from Plant Tissue

1) Grind Sample (up to 100 mg) using liquid nitrogen. Transfer the tissue powder to an appropriately sized tube and add **500 µl** of **RB Buffer** to a maximum of 100 mg tissue powder and vortex vigorously.
2) Incubate at **60℃** for **1-3 min**.
3) Centrifuge at full speed for **2 min**.
4) Transfer the supernatant to a new 1.5 ml tube.
5) Add **absolute ethanol (not provided)** by 0.5 volume of sample to the sample and mix immediately by using pipette.
6) Go to **step 3** of "RNA Extraction from Cultured Cell" in page 1 and follow the instructions accordingly.

Ⅴ RNA Extraction from Animal Tissue

1) Homogenize the sample (20~30 mg) with a homogenizer, place them in a new 1.5 ml tube, and add **500 µl** of **RB Buffer**.
2) Centrifuge the lysate for 3 min at full speed, and transfer the supernatant to a new 1.5 ml tube.
3) Add **200 µl** of **absolute ethanol (not provided)** and mix immediately by using pipette.
4) Go to **step 3** of "RNA Extraction from Cultured Cell" in page 1 and follow the instructions accordingly.

※ For more information, please visit www.bioneer.com and refer to the User Guide of this kit.