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Extraction of RNA from plant tissue using QIAGEN Kit

Table-top high-speed micro centrifuges CT15E/CT15RE and fixed angle rotors T15A61/T15A62

We would like to introduce an example of extraction of RNA from plant tissue using centrifugal filter “RNeasy Plant Mini Kit” (supplied by QIAGEN). You can extract high quality RNA by a centrifuge; it is more efficient and faster than other methods.

【Instruments】

Centrifuge:	Table-top high-speed micro centrifuge CT15E or CT15RE
Rotor:	T15A61 fixed angle rotor (2ml/1.5ml x 24 tubes) or T15A62 fixed angle rotor
Extraction kit:	RNeasy Mini Kit (supplied by QIAGEN)

“x g” setting is also available.
Equipped with a refrigerator.

Table-top high-speed micro centrifuge CT15RE

T15A61 angle rotor

【Operating Procedure】

Grind plant tissue to fine powder with liquid nitrogen using a pestle and a mortar.

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Put the ground powder in a 2-ml micro tube.

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Add 450 µl of Buffer RLT in the tube and vortex it vigorously.

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Incubate it at 56°C for 1 to 3 minutes.

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Put QIAshredder spin column in a new 2-ml micro tube. Pour the lysate into the QIAshredder spin column. Be careful not to pipette cell debris when pour lysate in the spin column.
*Cut the cap of the micro tube containing the spin column.

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Centrifuge it at 15,000 rpm (21,500xg) for 2 minutes at room temperature.

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Pour the filtrate into a new 2-ml micro tube.

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Add 0.5 time greater capacity of ethanol (96% (v/v) or higher) in the tube and mix it with a

pipette gently.

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Put the RNeasy spin column in a new 2-ml micro tube. Pour 650 µl of mixed solution into the RNeasy spin column.
*Cut the cap of the micro tube containing the spin column.

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Centrifuge it at 9,200 rpm (8,000xg) for 15 seconds at room temperature.

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Discard the filtrate.

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Add 700 µl of Buffer RW1 to the RNeasy spin column.

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Centrifuge it at 9,200 rpm (8,000xg) for 15 seconds at room temperature.

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Discard the filtrate.

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Add 500 µl of Buffer RPE to the RNeasy spin column.

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Centrifuge it at 9,200 rpm (8,000xg) for 15 seconds at room temperature.

↓

Discard the filtrate.

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Add 500 µl of Buffer RPE to the RNeasy spin column.

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Centrifuge it at 9,200 rpm (8,000xg) for 2 minutes at room temperature.

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Put the RNeasy spin column used in the above procedure in a new 1.5-ml micro tube.

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Add 30 to 50 µl of sterile distilled water in the RNeasy spin column.

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Centrifuge it at 9,200 rpm (8,000xg) for 1 minute at room temperature.

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Remove the RNeasy spin column from the tube. Preserve the eluted RNA solution in the tube at -70°C.

Note 1): Be sure to read the instruction manual of the rotor before use.
Note 2): Read the instruction manual of QIAGEN's kit for details of the protocol.
Note 3): **Do not spin the rotor over 10,000 rpm (12,000xg)** when using the 1.5-ml micro tube with the cap opened at 4°C. Otherwise, the cap will be torn.

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