Aurum™ Total RNA Mini Kit

Spin Format
Protocol Overview*

Cultured cells

Adherent
Rinse vessel with PBS, aspirate.

Lyse in vessel if # of cells <2 x 10^6.

Nonadherent
Rinse with PBS.

Transfer up to 2 x 10^6 cells, centrifuge 2 min.

Decant supernatant.

Add 350 µl lysis solution. Pipet up and down 12x.

Add 350 µl 70% EtOH. Pipet up and down.

Bacterial cells

Transfer up to 2.4 x 10^9 cells into a capped 2 ml tube. Centrifuge at maximum speed 1 min. Decant supernatant.

Add 100 µl of 500 µg/ml lysozyme. Pipet up and down.

Incubate at room temp. for 5 min.

Add 350 µl lysis solution. Pipet up and down 12x.

Add 250 µl 70% isopropyl alcohol. Pipet up and down.

Yeast cells

Transfer up to 3 x 10^7 cells into a capped 2 ml tube. Centrifuge at maximum speed 1 min. Decant supernatant.

Add 1 ml of 50 U/ml lyticase in lyticase dilution buffer. Pipet up and down.

Incubate at room temp. for 10 min. Centrifuge at 5,000 rpm for 5 min. Discard supernatant.

Add 350 µl lysis solution. Pipet up and down 12x.

Add 350 µl 70% EtOH. Pipet up and down.

Continue with the following steps for all sample types:

Insert RNA binding column into a 2 ml capless tube.

Transfer lysate.
Centrifuge 30 sec. Discard filtrate.

Add 700 µl low stringency wash.
Centrifuge 30 sec. Discard filtrate.

Dilute 5 µl reconstituted* DNase I with 75 µl DNase dilution solution.

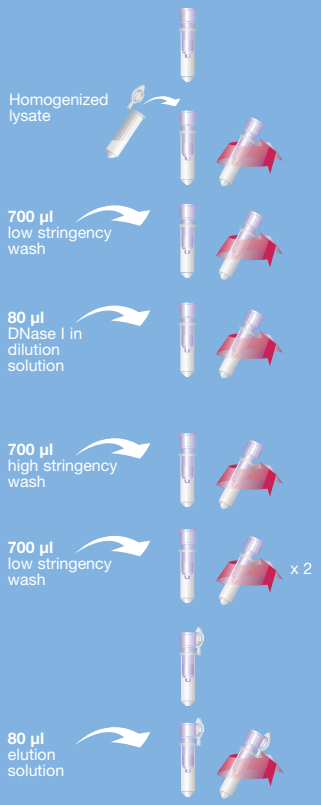
Add 80 µl diluted DNase I.
Incubate 15 min at room temp. Centrifuge 30 sec. Discard filtrate.

Add 700 µl high stringency wash.
Centrifuge 30 sec. Discard filtrate.

Add 700 µl low stringency wash.
Centrifuge 1 min. Discard filtrate. Centrifuge additional 2 min.

Place RNA binding column into a 1.5 ml capped tube.

Add 80 µl 70°C elution solution onto membrane stack.
Incubate 1 min. Centrifuge 2 min to elute.



* Refer to manual for detailed protocol.

Aurum Total RNA Mini Kit: Cat. #732-6820

BIO-RAD

For more information, call Technical Service at 1-800-4BIORAD (1-800-424-6723). Visit us on the Web at **www.bio-rad.com**

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Spin Format
Protocol Overview*

Animal tissue

Cut tissue into small pieces (<5 mm).

Grind into fine powder under liquid nitrogen.

Do not let tissue thaw.

Transfer up to 20 mg (hard tissue) or
40 mg (soft tissue) to a capped 2 ml tube.

Plant tissue

Cut tissue into small pieces (<5 mm).

Grind into fine powder under liquid nitrogen.

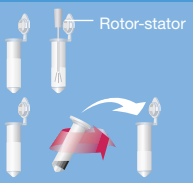
Do not let tissue thaw.

Transfer up to 60 mg to
a capped 2 ml tube.

Continue with the following steps for all sample types:

Add 700 µl lysis solution.
Disrupt vigorously with rotor-stator for 30–60 sec.

700 µl
lysis solution

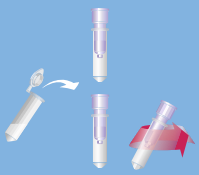


Centrifuge lysate at maximum speed 3 min.
Transfer supernatant to a new 2 ml capped tube.

Add 700 µl EtOH (60% EtOH for animal tissue, 70% EtOH for plant tissue) to supernatant.
Homogenize with rotor-stator 30 sec.

700 µl
60% EtOH
or
70% EtOH

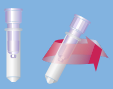
Insert RNA binding column into a 2 ml capless tube.



Transfer lysate, centrifuge 60 sec.
Discard filtrate. Repeat if necessary.

Add 700 µl low stringency wash.
Centrifuge 30 sec. Discard filtrate.

700 µl
low stringency
wash



Dilute 5 µl reconstituted* DNase I with 75 µl DNase dilution solution.

Add 80 µl diluted DNase I.
Incubate at room temp. 25 min for animal tissue, 15 min for plant tissue. Centrifuge column 30 sec. Discard filtrate.

80 µl
DNase in
dilution
solution

Add 700 µl high stringency wash.
Centrifuge 30 sec. Discard filtrate.

700 µl
high stringency
wash

Add 700 µl low stringency wash.
Centrifuge 30 sec. Discard filtrate.
Centrifuge additional 1 min.

700 µl
low stringency
wash

Place RNA binding column into a 1.5 ml capped tube.

Add 80 µl 70°C elution solution onto membrane stack.
Incubate 1 min. Centrifuge 2 min to elute.

80 µl
elution
solution

* Refer to manual for detailed protocol.