Aurum™ Total RNA Fatty and Fibrous Kit

Vacuum Format
Protocol Overview*

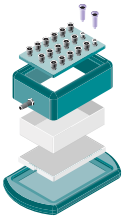
Fresh Tissue	Frozen Tissue	Cells Grown in a Monolayer	Suspension Cells
Immediately add 1 ml of PureZOL™ reagent to up to 100 mg of freshly dissected tissue.	Grind frozen tissue to a fine powder with a mortar and pestle under liquid nitrogen.	Aspirate the culture medium from a 10^2 cm plate containing cells grown in a monolayer.	Transfer up to 1 x 10^7 cells into a tube and spin for 2 min.
Disrupt and homogenize vigorously for 30–60 sec.	Do not let the tissue thaw.	Do not wash cells prior to adding PureZOL.	Do not wash cells prior to adding PureZOL.
Incubate lysate at room temp. for 5 min.	Add 1 ml of PureZOL to up to 100 mg of tissue.	Immediately add 1 ml of PureZOL directly in the culture dish and pipet up and down to lyse.	Immediately add 1 ml of PureZOL directly in the tube and pipet up and down to lyse.
	Disrupt and homogenize vigorously for 30–60 sec.	Incubate lysate at room temp. for 5 min.	Incubate lysate at room temp. for 5 min.
	Incubate lysate at room temp. for 5 min.		

Continue with the following steps for all sample types:

Add 0.2 ml of chloroform. Cover and shake vigorously for 15 sec. Incubate for 5 min at room temp.

Centrifuge at 12,000 x g for 15 min at 4°C. Carefully transfer only the aqueous phase to a new 2.0 ml tube.

Add an equal volume (approx. 600 µl) of 70% ethanol. Mix thoroughly by pipetting up and down.



Assemble manifold properly for isolation.

Transfer 700 µl of lysate.
Apply vacuum. Repeat with remaining lysate.

Add 700 µl low-stringency wash.
Apply vacuum.

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

Add 80 µl diluted DNase I.
Incubate 15 min at room temp. Apply vacuum.

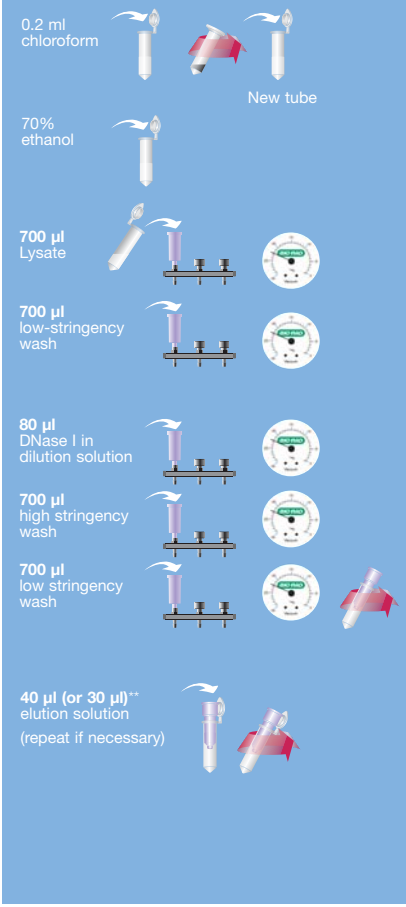
Add 700 µl high-stringency wash.
Apply vacuum.

Add 700 µl low-stringency wash.
Apply vacuum. Spin-purge 2 min into a 2 ml capless tube.

Place RNA binding column into a 1.5 ml capped tube.

Add 40 µl (or 30 µl) of 70°C elution solution onto the center of the membrane stack.**
Incubate 1 min. Centrifuge 2 min to elute.

Repeat the elution with another 40 µl of elution solution.**



* Refer to manual for detailed protocol.
** When isolating total RNA from small amounts of starting material (<10 mg of tissue or 500,000 cells), perform a single elution with 30 µl of warmed elution solution. Do not repeat the second elution.

BIO-RAD

For technical support, call your local Bio-Rad office, or in the US, call 1-800-4BIORAD (1-800-424-6723).
Visit us on the Web at **discover.bio-rad.com**

Aurum™ Total RNA Fatty and Fibrous Kit

Spin Format
Protocol Overview*

Fresh Tissue	Frozen Tissue	Cells Grown in a Monolayer	Suspension Cells
Immediately add 1 ml of PureZOL™ reagent to up to 100 mg of freshly dissected tissue.	Grind frozen tissue to a fine powder with a mortar and pestle under liquid nitrogen.	Aspirate the culture medium from a 10^2 cm plate containing cells grown in a monolayer.	Transfer up to 1 x 10^7 cells into a tube and spin for 2 min.
Disrupt and homogenize vigorously for 30–60 sec.	Do not let the tissue thaw.	Do not wash cells prior to adding PureZOL.	Do not wash cells prior to adding PureZOL.
Incubate lysate at room temp. for 5 min.	Add 1 ml of PureZOL to up to 100 mg of tissue.	Immediately add 1 ml of PureZOL directly in the culture dish and pipet up and down to lyse.	Immediately add 1 ml of PureZOL directly in the tube and pipet up and down to lyse.
	Disrupt and homogenize vigorously for 30–60 sec.	Incubate lysate at room temp. for 5 min.	Incubate lysate at room temp. for 5 min.
	Incubate lysate at room temp. for 5 min.		

Continue with the following steps for all sample types:

Add 0.2 ml of chloroform. Cover and shake vigorously for 15 sec. Incubate for 5 min at room temp.

Centrifuge at 12,000 x g for 15 min at 4°C. Carefully transfer only the aqueous phase to a new 2.0 ml tube.

Add an equal volume (approx. 600 µl) of 70% ethanol. Mix thoroughly by pipetting up and down.

Insert RNA binding column into a 2 ml capless tube.

Transfer 700 µl of lysate.
Centrifuge 30 sec. Discard filtrate. Repeat with remaining lysate.

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.

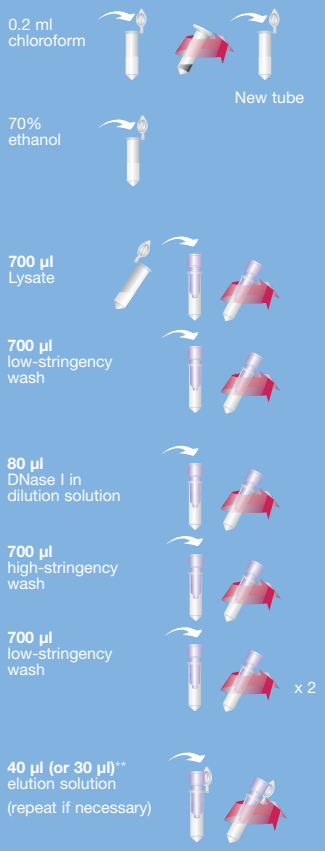
Add 700 µl high-stringency wash.
Centrifuge 30 sec. Discard filtrate.

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 40 µl (or 30 µl) of 70°C elution solution onto the center of the membrane stack.**
Incubate 1 min. Centrifuge 2 min to elute.

Repeat the elution with another 40 µl of elution solution.**



* Refer to manual for detailed protocol.

** When isolating total RNA from small amounts of starting material (<10 mg of tissue or 500,000 cells), perform a single elution with 30 µl of warmed elution solution. Do not repeat the second elution.

Aurum Total RNA Fatty and Fibrous Tissue Kit:
Cat. #732-6830