Aurum™ Total RNA 96 Kit — Protocol Overview*

Cultured cells

Adherent
Rinse vessel with PBS, aspirate.

Lyse in vessel if # of cells <1 x 10^6.

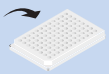
Nonadherent
Rinse with PBS.

Transfer up to 1 x 10^6 cells per microplate well.

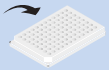
Centrifuge plate at 300 x g for 5 min.

Aspirate supernatant.

Add 150 µl lysis solution. Pipet up and down.



Add 150 µl 70% EtOH. Pipet up and down.



Bacterial cells

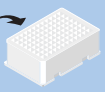
Transfer up to 8 x 10^8 cells into each well of a grow block.

Centrifuge at 1,500 x g for 10 min. Decant supernatant.

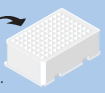
Add 100 µl of 500 µg/ml lysozyme. Pipet up and down

Incubate at room temp. for 5 min.

Add 350 µl lysis solution. Pipet up and down.



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



Yeast cells

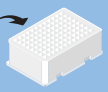
Transfer up to 2 x 10^7 cells into each well of a grow block.

Centrifuge at 1,500 x g for 10 min. Decant supernatant.

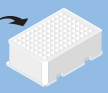
Add 1 ml of 50 U/ml lyticase in lyticase dilution buffer. Pipet up and down.

Incubate at room temp. for 10 min. Centrifuge at 1,500 x g for 5 min. Discard supernatant.

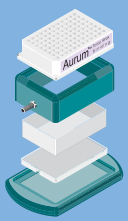
Add 350 µl lysis solution. Pipet up and down.



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



Continue with the following steps for all sample types:



Assemble manifold properly for isolation.

Transfer lysate to wells of RNA binding plate.
Apply vacuum.

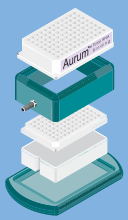
Add 700 µl low stringency wash to each well.
Apply vacuum.

For each well, dilute 2.5 µl reconstituted* DNase I with 77.5 µl DNase dilution solution.

Add 80 µl of diluted DNase I to each well.
Incubate 10 min at room temp. Apply vacuum.

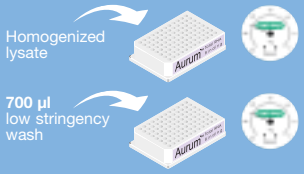
Add 700 µl high stringency wash to each well.
Apply vacuum.

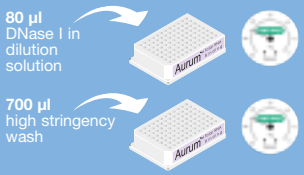
Add 700 µl low stringency wash to each well.
Apply vacuum.

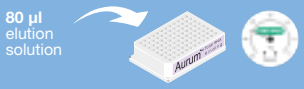


Assemble manifold properly for elution.

Add 80 µl 70°C elution solution per well onto membrane stack.
Incubate 1 min. Apply vacuum gradually to –20 to –23" Hg. Continue to apply vacuum for 5 min to elute.







* Refer to manual for detailed protocol.

Aurum Total RNA 96 Kit: Cat. #732-6800

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Bulletin 2925 US/EG Rev A

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