circRNA Isolation Kit

Cat. No. G4001

Store all components except RNA Purification Magnetic Beads at -20°C.
Store RNA Purification Magnetic Beads at 4°C.

Component	Quantity	Cat. No.
Poly(A) Polymerase, *E. coli*	25 µl	E099-1
10X Poly(A) Polymerase, *E. coli* Reaction Buffer	1.0 ml	E099-2
ATP (10 mM)	50 µl	E099-3
RNA Purification Magnetic Beads	5 ml	G971
RNase R	50 µl	E049-1
10X RNase R Reaction Buffer	1.0 ml	E049-2
RNaseOFF Ribonuclease Inhibitor	100 µl	G138

Description

abm's circRNA Isolation Kit allows for the enrichment of a novel class of RNA called circular RNA from a total RNA sample. Poly(A) Polymerase, *E. coli* catalyzes the addition of adenosine residues to the 3' termini of all linear RNAs – including structured RNAs. This results in RNAs exhibiting a long, 3' unstructured overhang which is optimally bound by RNase R. RNase R can then illicit 3' to 5' exonuclease activity resulting in improved degradation of linear RNAs which contributes to higher purity circRNAs.

Protocol

Additional Materials Required (not included)

- Freshly prepared 80% ethanol
- Nuclease-free water
- Magnetic separation rack

Note: Before starting, prepare 80% ethanol fresh prior to each experiment.

Part A – Poly(A) Addition

1. In a sterile tube, set up the following reaction and incubate at 37°C for 30 min using a thermal cycler.

Component	Volume
Total RNA	5 µg
10X Poly(A) Polymerase, *E. coli* Reaction Buffer	2 µl
ATP (10mM)	2 µl
Poly(A) Polymerase, *E. coli*	1 µl
Nuclease-free water	Up to 20 µl

2. Remove sample from thermal cycler and place on ice until the next step.

Part B – Total RNA Purification using Magnetic Beads

1. Remove RNA Purification Magnetic Beads from 4°C and incubate at room temperature for 30 min. Vortex the beads thoroughly for 30 s.
2. Bring sample volume from Part A to 50 µl by adding an appropriate amount of Nuclease-free water. Next, add 90 µl of RNA Purification Magnetic Beads (at a 1.8X bead to sample ratio) and pipette ten times to thoroughly mix. Incubate at room temperature for 10 min.
3. Place tube onto magnetic rack for 5 min or until the solution becomes clear.
4. Keep the tube on the magnetic rack; carefully remove the supernatant by pipette and discard.
5. Keep the tube on the magnetic rack; add 200 µl of freshly prepared 80% ethanol. Dispense the liquid onto the opposite side of the tube in order to avoid disturbing the beads. Incubate 30 s and then remove the supernatant by pipette. Repeat for a total of two washes.
6. Keep the tube on the magnetic rack; remove residual ethanol by pipette and then open tube caps to air dry for 2-5 min. Monitor the appearance of the beads closely.
   •Beads are ready for elution when they appear "matte." Beads that appear "shiny" still contain residual ethanol; beads that appear "cracked" have been over-dried. The latter two scenarios will result in contamination and reduced yield.
7. Add 23 µl of Nuclease-free water to the RNA Purification Magnetic Beads and remove the tube from the magnetic rack. Pipette the mixture thoroughly to resuspend. Incubate at room temperature for 5 min.
8. Place the tube on the magnetic rack for 5 min or until the solution becomes clear. Carefully remove the supernatant by pipette and transfer into a new sterile tube.
   •Ensure no bead carry-over. If beads become dislodged, simply mix the sample again and place tube back onto the magnetic rack for separation.
9. Place sample on ice until the next step.

Applied Biological Materials Inc. • 1-866-757-2414 • info@abmGood.com • www.abmGood.com

Part C – RNase R Degradation of Linear RNAs

1. In a sterile tube, set up the following reaction and incubate at 37°C for 2-3 h using a thermal cycler.

Component	Volume
Purified RNA from Part B	20 µl
10X RNase R Reaction Buffer	5 µl
RNaseOFF Ribonuclease Inhibitor	1.5 µl
RNase R	2 µl
Nuclease-free water	Up to 50 µl

2. Remove samples from thermal cycler and place on ice until the next step.

Part D – circRNA Purification using Magnetic Beads

1. Remove RNA Purification Magnetic Beads from 4°C and incubate at room temperature for 30 min. Vortex the beads thoroughly for 30 s.

2. Add 90 µl of RNA Purification Magnetic Beads (at a 1.8X bead to sample ratio) to sample from Part C and pipette ten times to thoroughly mix. Incubate at room temperature for 10 min.

3. Follow Steps 3-8 outlined in Part B.

4. Purified circRNA is ready for downstream applications or long term storage at -80°C.

General Notes

NGS Applications

- We recommend increasing the amount of Total RNA to 10 µg in Part A and increasing the amount of RNase R to 4 µl in Part C.

RNA Purification Magnetic Beads

- Store RNA Purification Beads tightly sealed at 4°C upon arrival. Do not freeze!
- Ensure beads come to room temperature (20-25°C) before use; using or storing beads at the incorrect temperature will result in lower yield and difficulty with handling.

RNase R

- Keep in mind that circRNAs represent a small proportion of total RNA (~0.1-0.01%), therefore RNase R treatment will most likely result in low levels of RNA, possibly undetectable by most methods.

Applied Biological Materials Inc. • 1-866-757-2414 • info@abmGood.com • www.abmGood.com