



RNA EXTRACTION FROM ANIMAL TISSUE USING THE PRECELLYS TISSUE TOTAL RNA EXTRACTION KIT

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CONTEXT

RNA extraction is a routine technique in many molecular biology laboratories. High quality RNA is a prerequisite to succeeding in a series of downstream analyses. Nonetheless, isolating RNA from biological samples can be a challenging procedure.

RNA is notoriously unstable and sensitive to temperature. High quality and high yield RNA can be obtained from any type of tissue sample thanks to an optimal extraction process that relies on 2 steps: tissue homogenization and RNA purification. Finding the appropriate protocols for each one of these steps, and combining them to obtain the best out of any tissue sample is a mandatory milestone for most laboratories.

With its wide range of tissue homogenizers, Bertin Instruments addresses the first challenge of tissue disruption by using a powerful 3D bead-beating technology under the Precellys® brand. Bertin Bioreagent has thus developed an RNA extraction kit to complete the sample prep workflow resulting in the Precellys Tissue RNA Extraction Kit, a solution for all of your genomic applications.

In this Application Note, we present the results obtained using the Precellys Tissue Total RNA Extraction Kit for the isolation and purification of RNA from mouse heart tissue, and how they compare to some of our competitors.

MATERIALS

- **Precellys Tissue Total RNA Extraction Kit** (Cat No. D05711)
- **Precellys 24 Touch** (Cat No. P002391-P24T0-A.0)
- **Precellys Lysing Kit CK28-R** (Cat No. P000916-LYSK0-A)
- 9 mouse heart samples (10 mg/ sample)
- RNA extraction kit Competitor Q
- RNA extraction kit Competitor M
- Ethanol (100 %)
- β -mercaptoethanol





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PROTOCOL

Tissue Homogenization & Lysis

Samples were preserved in RNAlater and conserved at -80 °C

- 1) Add sample to the lysing kit tube (CK28-R)
- 2) Add 350 µl RNA Lysis Buffer and 20 µl β-mercaptoethanol (optional). Vortex
- 3) Homogenize samples (Precellys 24 Touch - 6500 rpm for 3 x 10 sec)
- 4) Centrifuge (≥10,000 x g) for 5 minutes
- 5) Transfer the supernatant to a sterile 1,5 ml microcentrifuge tube

Bind -> Wash -> Elution, following the instructions from the Precellys Tissue Total RNA Extraction Kit user guide

RNA Quantification and Quality

- Quantification and quality analysis of total RNA by NanoDrop
- RT-qPCR of a housekeeping gene (B2M).

RESULTS

Mean RNA concentration obtained with the Precellys Tissue Total RNA Extraction Kit was 3711 ± 270 ng of RNA/mg of tissue for mouse heart samples.

RNA yield was almost twice the amount than that obtained with the RNA extraction kits from the competitors M and Q.

Table 1.- RNA concentrations (ng of RNA/mg of tissue) of mouse heart samples obtained with the Precellys Tissue RNA Extraction Kit (Bertin Bioreagent).

| Sample | RNA (ng/mg) | 260/280 | 260/230 |
|---------|-------------|---------|---------|
| Heart 1 | 3470,40 | 2,14 | 2,09 |
| Heart 2 | 4087,40 | 2,04 | 2,07 |
| Heart 3 | 3574,80 | 2,13 | 2,17 |

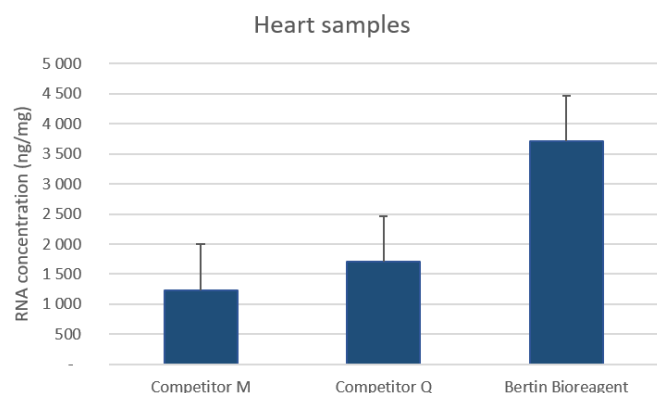


Figure 1.- RNA concentrations (ng of RNA/mg of tissue) of mouse heart samples obtained with the Precellys Tissue Total RNA Extraction Kit (Bertin Bioreagent), competitor M and competitor Q.



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The results obtained from the qPCR of the housekeeping gene B2M for mouse heart cDNA samples, showed an overall mean ΔCq value of 3.22, with a SD = 0.20 between 10-fold dilutions (Figure 2).

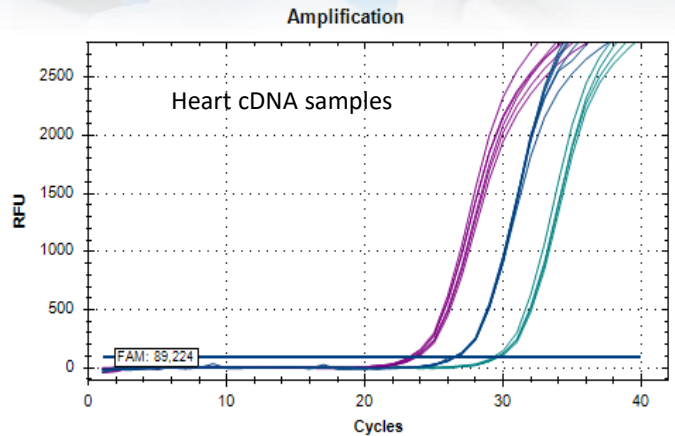
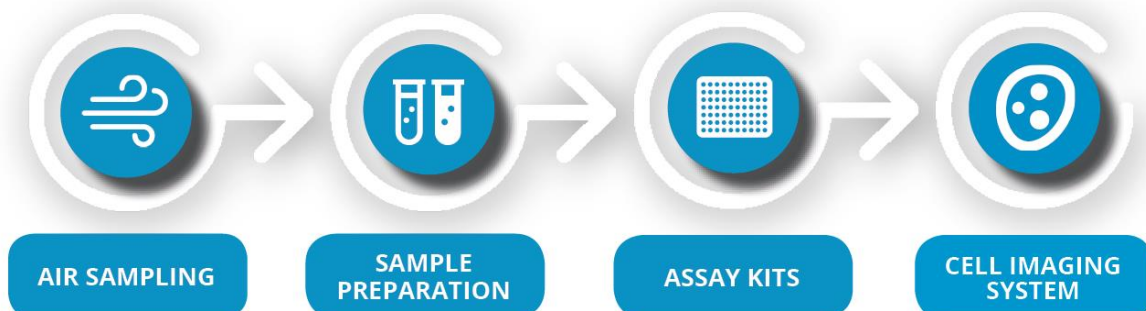


Figure 2.- qPCR results obtained for housekeeping gene B2M for heart cDNA samples after RNA extraction using the Precellys Tissue RNA Extraction Kit (Bertin Bioreagent).

CONCLUSION

The Precellys Tissue Total RNA Extraction Kit from Bertin Bioreagent allows to extract high quality RNA with a very high yield from diverse animal tissues. In conjunction with the power of the 3D bead-beating technology of the Precellys homogenizers, a complete solution is now available for the sample preparation workflow in biomolecular laboratories.

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