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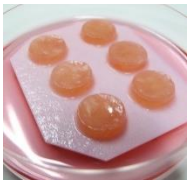
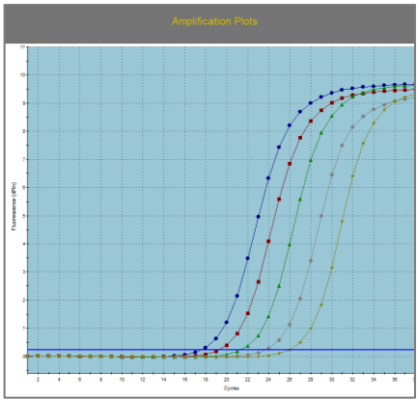
Phenion® FT Skin Model

RNA Isolation II

(Tissue lysis)





Preface

Reliable results in RNA-preparation are routinely achievable when using RNeasy® Mini Kits from Qiagen (Hilden, Germany). For safety instructions, please refer the manufactures instructions. Please note that other suppliers might also lead to excellent results.

This protocol – which is slightly adapted from the Qiagen RNeasy Mini Kit protocol – typically yield in 20-30 µg RNA, with an $OD_{260/280}$ ratio of $\geq$ 2.0, when processing one half of the Phenion® FT Skin Model. This yield suits most RNA analyses methods, enabling to subject the other half of the tissue to further analytical methods, e.g. histological assessments or protein analyses.

For maximum RNA-yields, we recommend to homogenize the tissue using a mixer mill and to follow the alternative protocol "Phenion® FT Skin Model RNA-Isolation **I**".

Materials/Disposables/Reagents

Material	Company	Order-No.
RNeasy Mini Kit	Qiagen, (Hilden, Germany)	74104
Proteinase K	Qiagen, (Hilden, Germany)	19131 or 19133
Disposable Scalpel	e.g. Braun (Tuttlingen, Germany)	5518059
1.5 ml / 2.0 ml Reaction tubes (PCR-grade, RNase free)	Eppendorf (Hamburg, Germany)	0030 123.328 0030 123.344
Centrifuge e.g. Biofuge fresco	Heraeus Kendro, (Osterode, Germany)	750 05521

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Thermomixer compact for 2 ml vials	Eppendorf (Hamburg, Germany)	5350 00836

Procedure

1. Dissect Phenion® FT Skin Model in two halves (store first half appropriately for further processing of your choice) and cut second half into eight equally sized pieces.

2. Transfer tissue pieces into 2 ml reaction tube and add 350 µl RLT buffer supplemented with 10 µl ß-Mercaptoethanol/ml buffer.

3. Place reaction tube in shaker for 45 min at RT.

4. Add 500 µl RNase free water and 10 µl proteinase K. Mix gently and incubate 10 - 60min at 55 °C in a Thermomixer compact.

5. Pellet cell debris by centrifugation at 8.000 x g for 3 min.

6. Pipet supernatant into a new collection tube and add 0.7 volumes of 98% ethanol. Mix gently by pipetting up and down slowly (do not vortex).

7. Transfer 700 µl onto RNeasy Mini Kit Spin Column. Centrifuge for 15 sec at 8.000 x g and discard flow-through.

8. Transfer remaining lysate onto corresponding spin column. Centrifuge for 15 sec at 8.000 x g and discard flow-through.

9. Wash spin column with 350 µl RW1 buffer. Centrifuge for 15 sec at 8.000 g and discard flow-through.

10. Add 80 µl DNase solution (10 µl DNase stock solution + 70 µl RDD buffer) to the spin column membrane and incubate 15 min at RT.

11. Add 350 µl RW1 buffer to the spin column. Centrifuge for 30 sec at 8.000 x g and discard flow-through.

12. Add 500µl RPE buffer to the spin column Centrifuge for 30 sec at 8.000 x g and discard flow-through.

13. Place spin column on a new 2 ml collection tube and add 500 µl RPE buffer. Centrifuge for 30 sec at 8.000 x g and discard flow-through.

14. Dry membrane of spin column by centrifugation for 4 min at max speed.

15. Place spin column carefully in a new 1.5 ml collection tube and add 50 µl RNase-free water on the surface of the membrane. Centrifuge for 1 min at ≥8.000 x g to elute the RNA and store appropriately.