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COMPETENCE IN SKIN PHYSIOLOGY
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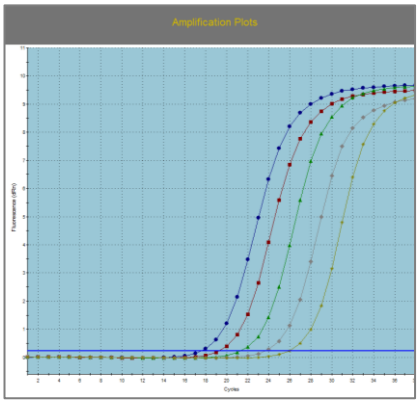
Phenion® FT Skin Model

RNA Isolation I

(Mechanical homogenization)



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.

For maximum RNA-yields, we recommend to homogenize the tissue using a mixer mill and to follow this protocol – which is slightly adapted from the Qiagen RNeasy Mini Kit protocol. If a mixer mill is not available, we recommend using the alternative protocol "Phenion® FT Skin Model RNA-Isolation **II**".

Typically, 25-40 µg RNA, with an $OD_{260/280}$ ratio of $\geq$ 2.0, is obtained from 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.

Materials/Disposables/Reagents

Material	Company	Order-No.
RNeasy Mini Kit	Qiagen, (Hilden, Germany)	74104
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
Mixer Mill e.g. Tissue Lyzer	Qiagen, (Hilden, Germany)	85300
Centrifuge e.g. Biofuge fresco	Heraeus Kendro, (Osterode, Germany)	750 05521

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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. Insert 5 mm stainless steel beats and close vial.

3. Disrupt tissue with a Mixer Mill for 5 min with agitation speed of 30 Hz

4. Pipet homogenate into a new tube. Add 500 µl deionized water and 10 µl proteinase K. Mix gently and incubate 10 min at 55 °C.

5. Pellet cell debris by centrifugation at 8.000 g for 30 sec.

6. Pipet supernatant into a new collection tube and add 0.5 volumes 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 500 µl RPE buffer. 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 repeat washing step with 500 µl RPE buffer.

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 3 min at 8.000 x g to elute the RNA and store appropriately.