SPLIT RNA Extraction Kit

Supplementary Protocol: Purification of RNA from FFPE Samples

Lexogen's SPLIT RNA Extraction Kit (Cat. No. 008) enables a fast and highly efficient extraction of RNA that is free of genomic DNA contamination. The RNA can be recovered from tissue, cells, fluids, and other sources as total RNA or split into a large and a small RNA fraction, facilitating the analysis of e.g., mRNA and miRNA from the same sample.
The isolation of RNA from Formalin-Fixed, Paraffin-Embedded (FFPE) tissue specimens with the SPLIT kit requires additional steps: Tissue Sample Preparation, Deparaffinization and Lysing of the Tissue, and Protein Digestion as described below.

User-supplied Consumables and Equipment

- For Deparaffinization and Lysing of the Tissue: heptane, methanol, 96 % ethanol.
- For Protein Digestion: 30 mM Tris, pH 8.0, Proteinase K (20 mg/ml).
- Benchtop centrifuge (12,000 - 16,000 rpm, rotor compatible with 1.5 ml micro-tubes).

Tissue Sample Preparation

1. Freshly cut sample sections with a thickness of 10 µm and a surface area of 0.5 - 1.5 cm^2. **ATTENTION:** Thicker sections may impair the quality of the RNA isolation and result in lower yield.
2. Place the cut sections in a 1.5 ml tube. Close and store until Deparaffinization at 0 °C - 10 °C. **REMARK:** Depending on the surface size and the age of the sample, we recommend using 3 - 10 sections per extraction.

Deparaffinization and Lysing of the Tissue

3. Quickly spin down the tube(s) containing the sections.
4. Add 500 µl heptane per tube and vortex for 10 seconds.
5. Incubate at room temperature for 10 minutes and vortex at least twice during incubation.
6. Add 25 µl methanol per tube and vortex for 10 seconds.
7. Centrifuge at 12,000 - 16,000 rpm for 2 minutes. Carefully remove and discard the supernatant.
8. Add 1 ml 96 % ethanol and vortex for 10 seconds.
9. Centrifuge at 12,000 - 16,000 rpm for 2 minutes. Carefully remove and discard the supernatant.
10. Open the tube cap and let the pellet air-dry for 10 minutes.

Protein Digestion

11. Resuspend the pellet in 80 µl 30 mM Tris, pH 8.0.
12. Add 20 µl Proteinase K (20 mg/ml) to each tube and mix by vortexing.
13. Incubate for 1 hour at 60 °C with occasional mixing (vortexing every 10 minutes, or using a thermomixer set to 300 rpm). **OPTIONAL:** Extending the incubation time up to 3 hours, or until any visible tissue debris has disappeared can improve yields.

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14. Proceed **immediately** with SPLIT by adding 400 µl Isolation Buffer (**IB**) and continue with the SPLIT RNA Extraction protocol for total RNA Extraction (see 5.2. Phenol-Chloroform Extraction, step 8, p.14 of the SPLIT RNA Extraction Kit User Guide: 008UG005).

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Fractions for pure RNA sequencing