LEXOGEN

Enabling complete transcriptome sequencing

SPLIT RNA Extraction Kit: Pure RNA Fractions for Demanding Applications

The SPLIT RNA Extraction Kit 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 obtained RNA is ideal for demanding applications such as Next Generation Sequencing library preparation, full-length cDNA generation, RT-PCR, or microarray analysis.

The SPLIT Workflow

The protocol is designed to be completed in 30 - 35 minutes and starts with sample homogenization in a highly chaotropic buffer that also readily dissolves solid tissues (Fig. 1). Cell debris, proteins and DNA are then separated from the RNA in an acidic phenol-chloroform extraction. This step is aided by the use of phase lock gel tubes, significantly facilitating the handling and increasing safety, separation and recovery. The organic extraction together with the ensuing silica-column based purification ensures an isolation of RNA without significant genomic DNA background (Fig. 2). Importantly, the RNA can be eluted either as total RNA covering the complete size range or split into two fractions. A double purification allows for the separate elution of the large RNA and the small RNA fractions with a cut-off at ~150 nt (Fig. 2). These fractions are ideally suited for the evaluation of RNA populations that require specific analysis pathways such as mRNAs, lncRNAs and miRNAs.

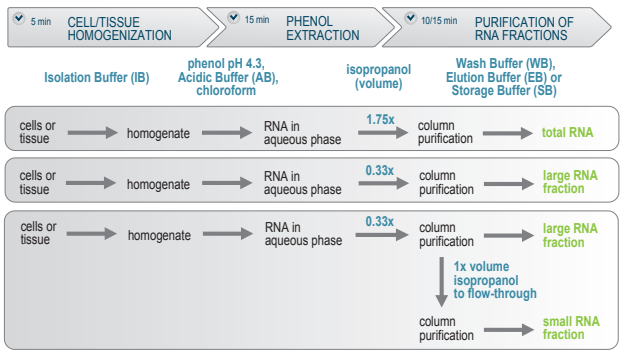

Figure 1 I Schematic overview of the SPLIT workflow. The cut-off between the large and small RNA fraction is at ~150 nt.

gDNA Removal and RNA Integrity

No further gDNA removal processes are necessary, preserving the extracted RNA. Other methods designed to control gDNA contamination mostly rely on enzymatic removal, whereby the application itself or the enzyme inactivation (e.g., by heat denaturation) can severely compromise RNA integrity. Similarly, size-filtration based methods such as gDNA removal columns result in either ineffective gDNA removal or exclusion of longer RNA molecules (Fig. 3).

Ordering Information

Catalog Numbers:
008 (SPLIT RNA Extraction Kit)
037 (RiboCop rRNA Depletion Kit)
039 (Poly(A) RNA Selection Kit)

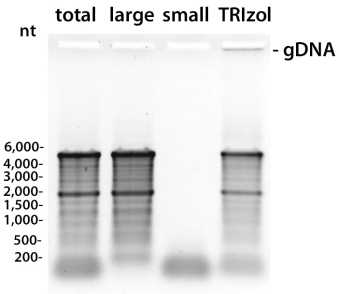

Figure 2 I The SPLIT protocol extracts RNA free of genomic DNA contamination and enables splitting into a large and small RNA fraction with a cut-off at ~150 nt. Extracted mouse liver RNA (total, large and small fraction) is shown on a denaturing formaldehyde agarose gel. A TRIzol-extracted control sample shows a significant amount of genomic DNA in this analysis.

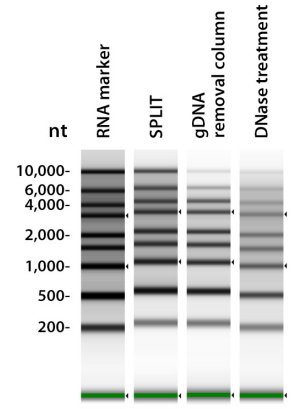

Figure 3 I The SPLIT protocol preserves RNA integrity and recovers the complete RNA size range without gDNA-removal associated adverse effects. RNA integrity of a transcript RNA marker (200 – 10,000 nt) was assessed on Agilent's TapeStation after employing different gDNA removal methods.

Key features of the SPLIT RNA Extraction Kit

- **Extraction of total RNA:** complete RNA size range from < 17 nt to > 10,000 nt
- **Splitting option:** split into large RNA and small RNA fractions with a cut-off at ~150 nt
- **Highest RNA integrity and purity:** RIN up to 10 for cell culture and RIN 8.0 - 9.7 for tissue samples (depending on the input); pure, intact full-length RNAs suitable for virtually all downstream applications
- **RNA free of genomic DNA:** no RNA degradation due to DNase treatment, no size bias caused by gDNA removal columns
- **High yield and extraction efficiency:** column binding capacity of up to 100 µg RNA; high efficiency even with input as low as 0.5 mg of tissue or 100 cells
- **Species-independent RNA extraction:** animal (e.g., mouse, human) and plant tissues (e.g., A. thaliana, Picea abies), insects (e.g., drosophila), cell lines (e.g., human), fluid samples (e.g., plasma), and others (e.g., jellyfish, fungi, bacteria)
- **Convenient and fast RNA extraction:** phase-lock gel tubes for efficient, comfortable, and safe phase separation; RNA in only 30 - 35 minutes

Extraction Efficiency and RNA Quality

The homogenization step of the SPLIT workflow is highly efficient and designed to obtain RNA of highest integrity (Table 1). The RNA quality almost exclusively depends on the quality of the input. A RIN of 10 can be easily obtained, from, e.g., freshly harvested cells, and RNA from tissue preserved with an RNA stabilizing reagent routinely yields a RIN of 8.0 – 9.7. The workflow can be adapted to suit a wide range of input materials (cell culture, animal and plant tissues, whole organisms such as bacteria, yeast or jellyfish, fluids such as blood, plasma and urine, etc.), and Lexogen strives to continuously expand this range with protocols given in the User Guide and online at www.lexogen.com.

Organism	Source	Total RNA		Large RNA		Small RNA
		Yield	RIN	Yield	RIN	Yield
Human	Cell culture	-	-	7.8 µg[1]	10.0	-
Mouse	Liver	4.1	8.9	3.0	8.8	0.8
	Brain	1.5	8.6	1.1	8.4	0.3
	Lung	2.4	8.7	2.0	9.0	0.5
	Heart	1.9	8.3	1.1	8.2	0.3
	Muscle	0.9	8.9	0.6	8.8	0.2
	Skin	1.6	8.9	0.8	9.0	0.1
A. thaliana	Seedling	-	-	3.1 µg[2]	8.2	1.9 µg[2]

Table 1 I Yield and integrity of RNA extracted with the SPLIT kit. RNA samples were assessed by photometry and microfluidics. Yield and RIN values (RNA Integrity Number calculated by Agilent's Bioanalyzer software) are the average of at least duplicates. Tissue / cells were stored in RNAlater (Life Technologies), homogenization was in a tissue grinder and yield is given in µg RNA / mg tissue except: [1]yield in µg RNA / 10^6 cells, [2]homogenization in liquid N_2; yield in µg / seedling.

miRNA- and mRNA-specific NGS Library Preparations from the Same Sample

Next Generation Sequencing of transcripts (RNA-Seq) has become the showcase of transcriptome research with its own special input RNA requirements. Samples extracted with the SPLIT kit deliver the whole range of RNA sizes for RNA-Seq, from miRNAs to mRNAs of over 10,000 nt length. Efficient recovery of siRNA and miRNA down to 17 nt in the total RNA or in the small RNA fraction has been shown in spike-in experiments with small RNA markers (Fig. 4).

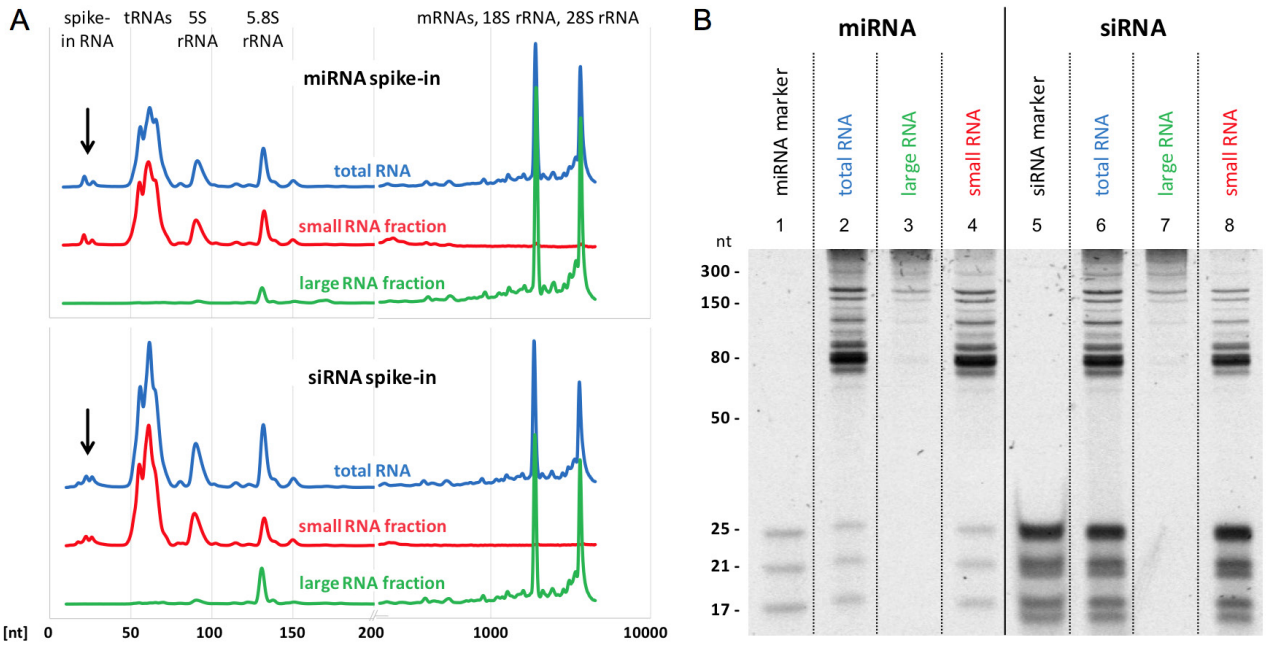

Figure 4 I miRNA-sized RNAs are efficiently recovered in the total RNA elution and in the small RNA fraction with the SPLIT kit. Mouse liver homogenate was spiked with a single-stranded miRNA marker or a double-stranded siRNA marker. Total RNA or small RNA and large RNA fractions were extracted with the SPLIT RNA Extraction Kit. (A) The RNA samples were analyzed on an Agilent Bioanalyzer on a small RNA chip (10 - 200 nt, linear scale) and on an RNA 6000 pico chip (200 - 5000 nt, log scale). The combination of the traces is shown for illustrative purposes; the Y-axes do not correspond quantitatively. (B) Polyacrylamide gel analysis of RNA samples. The theoretical maximum spike-in RNA recovery amount was loaded in lane 1 and lane 5, respectively, to enable a semi-quantitative comparison.

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SPLIT™
Fractions for pure RNA sequencing