xcelris genomics
An Abellon Company

XcelGen | Fungal RNA Mini Kit

User Guide

Cat No: XG6618-01

XcelGen

Ver.: 0.2/13

Revised Protocol

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Introduction

The Fungal RNA Mini Kit provides an easy and fast method for isolating RNA from Plant tissue within 30 min. Only trace genomic DNA exists in the purified RNA, which can be eliminated by DNase I treatment when it is necessary.

Overview

If using the Fungal RNA Mini Kit for the first time, please read this booklet to become familiar with the procedures. Samples are homogenized and lysed in a high salt buffer. The RNA is bound to the column while DNA, proteins and other impurities are removed by wash buffer.

Storage and Stability

All components of the Fungal RNA Kit are stable for at least 12 months when stored at 22°C-25°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer RLY. It is possible to dissolve such deposits by warming the solution at 50°C, though we have found that they do not interfere with overall performance.

Kit Contents

Product	XG6618-00	XG6618-01
Preps	4	50
Collection Tubes	8	100
Buffer RLY	3 ml	30 ml
Buffer RB	2 ml	20 ml
RNA Wash Buffer*	1.2 ml	24 ml
DEPC-Treated H_2O	1 ml	5 ml
RNA Columns	4	50
DNA Clearance Column	4	50
1.5ml RNase-free microfuge tube	4	50
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Before Starting

- Add 4.8 ml (XG6611-00) or 96 ml (XG6611-01) 100% ethanol to each RNA Wash Buffer before use.
- Add 3 ml (XG6611-00) or 30 ml (XG6611-01) 100% ethanol to each Buffer RB before use.

Protocol for Extracting Total RNA From Fungal Tissue

Note: *Perform all steps including centrifugation at room temperature*

1. Weigh **50mg** Fungal tissue (no more than 100 mg) in a 2 ml tube. Freeze the Fungal tissue in liquid nitrogen and grind using a rotor starter.
2. Transfer **5 volume (500µl) Buffer RLY/β-mercaptoethanol** to the tube containing the Fungal tissue immediately. Grind using a rotor starter again.

   Note: *Ensure that β-mercaptoethanol has been added before use.* ***10µl β-mercaptoethanol*** *should be added in* ***500µl RLY.***
3. Transfer the cleared lysate to a DNA Clearance column pre-inserted in a 2 ml Collection tube. Centrifuge at 13,000 rpm for 2 min. Discard the DNA Clearance column and save the flow-through.

Note: *This step is for genomic DNA removal.*

4. Add **1 volume 100% ethanol** to the lysate (for example: 500µl 100% ethanol for 500µl lysate).
5. Transfer the solution into a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column back to a new collection tube.
6. Add **400µl Buffer RB** to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through.
7. Add **650µl RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.

   Note: *Ensure that ethanol has been added to RNA Wash Buffer before use.*
8. Add another **650µl RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through and collection tube, put the column, with the lid open, back to a new collection tube.
9. Centrifuge at 13,000 rpm for 5 min. Discard the flow-through.

   Note: *The residual ethanol will be removed more efficiently with the column lid open during centrifugation.*
10. Place the column to a RNase-free 1.5 ml tube, add **30-50 µl DEPC-treated ddH_2O** to the column,incubate at room temperature for 1 min, and centrifuge at 13,000 rpm for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20°C. Reload the eluted RNA solution to the column and centrifuge at 13,000 rpm again.

    Note: *It is highly recommended that RNA quality to be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to the RNA column. An A_{260}/A_{280} ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.*

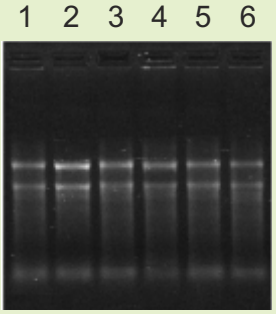

Fig: Denaturing Agarose gel analysis of RNA isolated from different Fungal tissues with XcelGen Fungal RNA mini Kit.

Troubleshooting Guide

Problems	Possible Reasons	Suggestion
Low A_{260}/A_{280} ratio	Protein contamination	Do a Phenol: Chloroform extraction. Loss of total RNA (up to 40%) should be expected.
Low Yield	RNA in sample degraded	Freeze samples immediately in liquid nitrogen and store at -70°C after collect it.
Low Yield	The binding capacity of the membrane in the spin column was exceeded	Use of too much tissue sample exceeding the binding capacity of spin column will cause the decreasing of total RNA yield.
Low Yield	Ethanol not added to Buffer	Add ethanol to the RNA Wash Buffer and Buffer RW before purification.
Genomic DNA contamination	Too much total RNA sample was used in RT-PCR.	Reduce total RNA amount used in RT-PCR to 50-100 ng.
Genomic DNA contamination	The sample may contain too much genomic DNA.	Reduce the amount of starting tissue in the preparation of the homogenate. Most tissues will not show a genomic DNA contamination problem at 30 mg or less per prep.

Limited Use and Warranty

This product is intended for in vitro research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in XcelGen's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by XcelGen. XcelGen's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of XcelGen, to replace the products, XcelGen shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or for more product information, please visit our website at **www.xcelrisgenomics.com**

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Product & Services

XcelGen

Quality Kits made by Xperts

• Plasmid DNA Isolation Kits • Genomic DNA Extraction Kits • RNA Extraction Kits • Polymerase • DNA Ladders • DNA Markers
• Premix Taq • dNTP's • RAPD kits • Agarose • Glycerol • Tms NA Stabilizers & RNA Protectant solutions

PrimeX

Oligo Synthesis & Purification Services

• 10 nmole • 25 nmole • 50 nmole • 100 nmole • 200 nmole • 1000 nmole

NXT

NGS Services

• Denovo Genome Sequencing • Whole Genome Resequencing • GBS/RAD Sequencing • Exome Sequencing • Amplicon Sequencing
• Whole Transcriptome Analysis/RNA-Sequencing • Small RNA Sequencing • Metagenomics • Metatranscriptomics
• ChIP Sequencing • Mitochondrial Sequencing • Next Generation Genomic Services on Illumina MiSeq
• Genotyping by Sequencing • Tilling/Ecotilling using NGS • Genome Database development Services

NGS Bioinformatics

• In silico Primer Design • Microarray Analysis • Metagenomics • Physical, Genetic and QTL mapping
• Assembly and annotation of prokaryotic and eukaryotic genome • Genome Mapping and SNP discovery
• Transcriptome discovery and analysis • sRNA analysis and discovery

XcelSeq

Sanger Sequencing Services

• Plasmid /PCR Sequencing Services • r-E. coli Culture Sequencing Services • Primer Walk Sequencing Services
• Microbial Identification Service • Multilocus Sequence Typing

Customised Services

• SNP Genotyping by SNaPshot Assay • Microsatellite Genotyping • Golden Gate Assays and Arrays
• Gene Expression on Real Time PCR • Gene expression on Agilent / Microarray / Affymetix • Library construction

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