ZYMO RESEARCH

RNA Purification Made Simple™

Pinpoint™ Slide RNA Isolation System I

RNA from any glass slide tissue section

Highlights

- Spin-column purification of total RNA from tissue mounted on glass slides.
- Pinpoint™ tissue sampling technology is combined with a one-step RNA extraction method.
- RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

Catalog Numbers:
R1003

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

Pinpoint™ Slide RNA Isolation System I	R1003 (50 prep)
Pinpoint™ Solution	1 ml
RNA Extraction Buffer	12 ml
RNA Wash Buffer[1] (concentrate)	6 ml
DNase/RNase-Free Water	1 ml
Zymo-Spin™ IC Columns	50
Collection Tubes	50
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

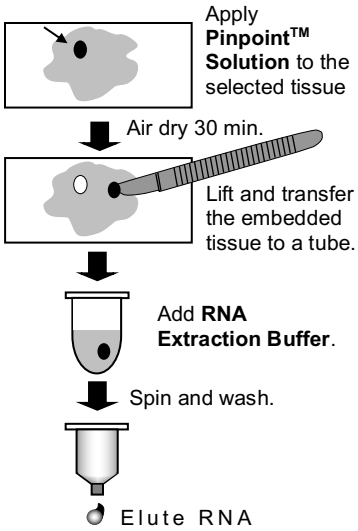
Specifications

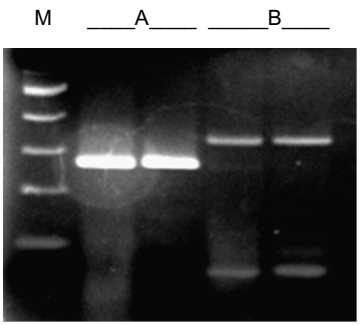
- **Sample Sources –** Cells from fresh or frozen tissue sections fixed glass slides[1] by ethanol, acetone, methanol, etc. Use an area of 1 to 100 mm^2 fresh tissue with 10 µm thickness (approximately 500-1000 cells, depending on the tissue type and cell density).
- **Size –** Total RNA including small/microRNAs (≥ 17 nt).
- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.
- **Binding Capacity – Zymo-Spin™ IC Column** yield up to 10 µg RNA.
- **Elution Volume –** ≥ 6 µl **DNase/RNase-Free Water.**
- **Equipment Needed (user provided) –** Microcentrifuge, vortex, incubator, autoclave.

1 For paraffin embedded tissue sections, use the Pinpoint™ Slide RNA Isolation System II (R1007).

Product Description

The **Pinpoint™ Slide RNA Isolation System I** is an innovative product designed to isolate RNA from any targeted area of a tissue on microscopic slides. The system combines a powerful **Pinpoint™** tissue sampling method with a unique, single-step RNA extraction/binding buffer that includes **Zymo-Spin™** column technology to yield high quality purified RNA. **Pinpoint™ Slide RNA Isolation System I** allows for the efficient recovery of RNA from fresh tissue sections for any subsequent RNA analyses including RT/qPCR.





RT/qPCR of human tissue section RNA recovered from tissue using the **Pinpoint™ RNA Isolation System I**. Duplicate samples are PCR products from A) human β-actin transcript B) an arbitrary human chromosome 3 transcript.

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) Total RNA Purification.

(I) Buffer Preparation

- ✓ Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

(II) Sample Preparation

Preparation of Tissue Sections

1. Clean glass sample slides with sterile ethanol/water and dry by autoclaving (or baking) at 300°C for 4 hours.
2. Mount tissue section (≥ 10 µm thick) onto a glass slide and dry it at 60°C for 30 minutes.
3. To fix the section, submerge the slide in 95% ethanol for 60 minutes.
4. Air dry the sample on the slide for approximately 30 minutes. Proceed to Pinpoint Fractionation, below.

Pinpoint Fractionation (to remove a selected area of tissue from a glass slide)

1. Using a sterile pipette tip, apply 0.5 µl of **Pinpoint™ Solution**[1] per mm^2 of tissue on the slide and gently spread the thick solution over the selected tissue region[1].
2. Allow the **Pinpoint™ Solution** to dry completely as a blue film at room temperature (typically 30-45 minutes), embedding the tissue and cells underneath.
3. Using a sterile blade or scalpel, cut and remove the embedded tissue section from the slide. Then transfer into a nuclease-free tube (not provided).
4. Centrifuge briefly to locate the tissue sample to the bottom of the tube. Proceed to Total RNA Purification, page 6.

1 Use an area of 1 to 100 mm^2 fresh tissue with 10 µm thickness (approximately 500-1000 cells, depending on the tissue type and cell density).

(III) Total RNA Purification

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 1 minute, unless specified.

1. Add 200 µl **RNA Extraction Buffer** to lyse the tissue sample and pipette up/down several times. Then vortex briefly.

2. Incubate the sample on ice for 30 minutes, vortexing briefly every 10 minutes.

3. Add 200 µl ethanol (100%) to the sample, mix and then incubate on ice for 10 minutes.

4. Transfer the mixture into a **Zymo-Spin™ IC Column**[1] in a **Collection Tube** and centrifuge. Discard the flow-through.

5. Add 200 µl **RNA Wash Buffer** and centrifuge the column to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).

6. Add 10 µl **DNase/RNase-Free Water**[2] directly to the column matrix and centrifuge.

   Alternatively, for highly concentrated RNA use ≥ 6 µl elution.

   The eluted RNA can be used immediately or stored frozen.

1 To process samples > 700 µl, columns may be reloaded.
2 To maximize RNA yield, increase the elution volume and/or repeat the elution.

Ordering Information

Product Description	Catalog No.	Size
Pinpoint™ Slide RNA Isolation System I	R1003	50 preps.

Individual Kit Components	Catalog No.	Amount
Pinpoint™ Solution	D3001-1	1 ml
RNA Extraction Buffer	R1003-2-12 R1003-2-50	12 ml 50 ml
RNA Wash Buffer (concentrate)	R1003-2-6 R1003-3-12	6 ml 12 ml
DNase/RNase-Free Water	W1001-6 W1001-10	6 ml 10 ml
Zymo-Spin™ IC Columns	C1004-50	50
Collection Tubes	C1001-50	50

Complete Your Workflow

✓ For tough-to-lyse samples, use ZR BashingBead Lysis Tubes:

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	Plant/animal tissue
0.1 + 0.5 mm beads #S6012	Microbes
0.1 + 2.0 mm beads #S6014	Microbes in tissue/insects

✓ For isolation of RNA from any sample:

Quick-RNA kits	
Miniprep Plus #R1057/R1058	≤ 10^7 cells, ≤ 50 mg tissue
MagBeads #R2132/R2133	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kits	
Microprep #R1013-R1014	DNase I Set included
MagBeads #R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
RNA degradation	RNA is very susceptible to RNase digestion; thus we encourage the use of freshly prepared tissue sections. If a sample cannot be processed immediately, store it at ≤-70°C or submerge it in a 95% ethanol bath at -20°C. Processing of tissue sections stored for ≥ 1 month at room temperature is not recommended. If the eluted RNA will not be used immediately it is recommended that 1 U/10 µl of RNase inhibitor be added to the sample prior to storage at -70°C.
Insufficient RNA	Make sure an appropriate sampling area is selected for processing. Select an area of the tissue that will contain ≥ 50 cells. Increase the sampling area if the tissue type contains few cells (e.g., fatty tissue and connective tissue). The sampling size can vary from 1 mm2 to over 100 mm2. We recommend that the sample thickness be ≥10 µm.
RT/qPCR parameters are not optimized	It is recommended that the conditions used for RT/qPCR be optimized prior to using template RNA purified by the **Pinpoint™ Slide RNA Isolation System I**. It may be necessary to increase both the annealing and extension times and adjust the number of cycles for low copy number mRNAs.
DNA contamination	To remove DNA: - Perform DNase I treatment post-purification. See RNA Clean & Concentrator kit #R1013.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

Notes

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