ZYMO RESEARCH

RNA Purification Made Simple™

# Pinpoint™ Slide RNA Isolation System II

RNA from any glass slide tissue section

## Highlights

- Spin-column purification of total RNA from paraffin-embedded tissue sections mounted on glass slides.
- Pinpoint™ tissue sampling technology is combined with a one-step RNA extraction method that includes Proteinase K for efficient lysis and homogenization.
- RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

Catalog Numbers:
R1007

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

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# Table of Contents

# Product Contents

| Pinpoint™ Slide RNA Isolation System II | R1007 (50 prep) |
|---|---|
| Pinpoint™ Solution | 1 ml |
| Proteinase K[1] (& Storage Buffer Set) | 5 mg |
| RNA Digestion Buffer | 1.2 ml |
| RNA Extraction Buffer | 3 ml |
| RNA Wash Buffer[2] (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 260 µl **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 5 mg. Mix and store frozen aliquots.
2 Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

# Specifications

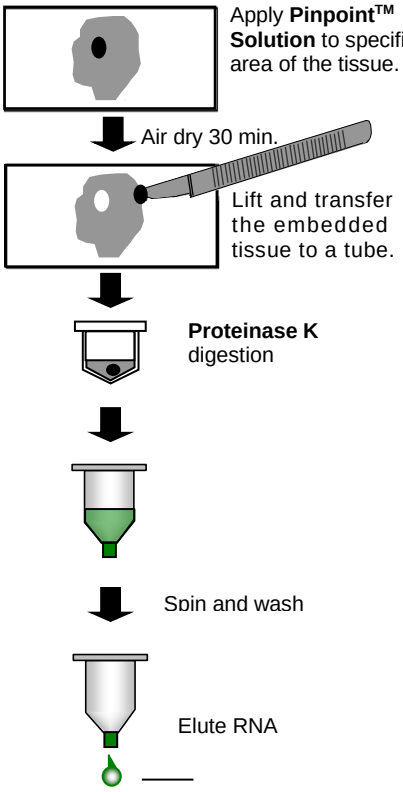
- **Sample Sources –** Cells from paraffin-embedded tissue[1] sections on glass slides. Use an area of 1 to 100 $mm^2$ fresh tissue with 10 µm thickness (approximately 500-1000 cells, depending on the tissue type and cell density).
- **Size –** Total RNA including small/microRNAs (≥ 17 nt).
- **Purity –** $A_{260}/A_{280}$ & $A_{260}/A_{230}$ > 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/water bath.

1 For fresh or frozen tissue sections, use the Pinpoint™ Slide RNA Isolation System I (R1003).

# Product Description

The **Pinpoint™ Slide RNA Isolation System II** is an innovative product designed to isolate RNA from any targeted area of a tissue on microscopic slides.

Simply apply the **Pinpoint™ Solution** to a selected area of tissue on a glass slide. The solution will air-dry forming a thin blue film that embeds the tissue underneath. The film is then lifted from the slide and treated with **Proteinase K**, **RNA Digestion Buffer** and **RNA Extraction Buffer**. The extracted RNA is then washed and concentrated using a **Zymo-Spin™ IC Column**. Eluted RNA can be used for any subsequent analysis including RT/qPCR.



# Protocol

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

## (I) Buffer Preparation

- ✓ Add 260 µl **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 5 mg. Mix and store frozen aliquots.
- ✓ Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

## (II) Sample Preparation

### Paraffin Removal from Tissue Samples

1. Mount the paraffin-embedded tissue section (≥ 10 µm thick) onto a glass slide and dry it at 60°C for 30 minutes.
2. Submerge the slide in xylene at room temperature for 30 minutes. Then change out/replace with fresh xylene and continue to submerge the slide for another 30 minutes.
3. Hydrate the sample by washing progressively for 2 minutes in 100%, 70%, 50% ethanol, and then with pure water.
4. Air dry the sample on the slide. 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 20 µl **RNA Digestion Buffer** and 5 µl **Proteinase K** to the tissue sample, mix gently and incubate at 55°C for 4 hours.

2. Add 50 µl **RNA Extraction Buffer** (2:1) and mix.

3. Add 75 µl ethanol (95-100%) (1:1) and mix.

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 of pre-warmed (60°C) **DNase/RNase-Free Water**[2] directly to the column matrix, let it stand for 2 minutes and then 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 II** | R1007 | 50 preps. |

| Individual Kit Components | Catalog No. | Amount |
|---|---|---|
| Pinpoint™ Solution | D3001-1 | 1 ml |
| RNA Extraction Buffer | R1003-2-12<br>R1003-2-50 | 12 ml<br>50 ml |
| RNA Wash Buffer (concentrate) | R1003-2-6<br>R1003-3-12 | 6 ml<br>12 ml |
| DNase/RNase-Free Water | W1001-6<br>W1001-10 | 6 ml<br>10 ml |
| Zymo-Spin™ IC Columns | C1004-50 | 50 |
| Collection Tubes | C1001-50 | 50 |
| RNA Digestion Buffer | R1007-1 | 1.2 ml |
| Proteinase K (& Storage Buffer Set) | D3001-2-5<br>D3001-2-20 | 5 mg<br>20 mg |

# 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:<br>- 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

# Notes

# Notes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This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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