ZYMO RESEARCH

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

# Pinpoint™ Slide RNA Isolation System I

Catalog No. **R1003**

## Highlights

- Allows for the isolation of total RNA from tissue mounted on glass slides.
- Simple procedure combines Pinpoint™ tissue sampling technology with a one-step RNA extraction/purification method.
- Isolates RNA that is suitable for use in RNA-based procedures including RT-PCR.
- Omits the use of organic denaturants as well as proteinases.

## Contents






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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

## Product Contents

| **Pinpoint™ Slide RNA Isolation System I** (Kit Size) | **R1003** (50 Preps.) | **Storage Temperature** |
|---|---|---|
| **Pinpoint™ Solution** | 1 ml | Room Temp. |
| **RNA Extraction Buffer** | 12 ml | Room Temp. |
| **RNA Wash Buffer**[1] (concentrate) | 6 ml | Room Temp. |
| **DNase/RNase-Free Water** | 1 ml | Room Temp. |
| **Zymo-Spin™ IC Columns** | 50 | Room Temp. |
| **Collection Tubes** | 50 | Room Temp. |
| **Instruction Manual** | 1 | - |

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

## Specifications

- **Sample Sources** – Cells from tissue sections on glass slides[2].
- **RNA Purity** – High quality RNA is recovered in RNase-free water. Some DNA may be present in the final preparation of RNA but can be removed using the **DNA-Free RNA Kit™** (R1013 & R1014).
- **RNA Recovery** – The RNA binding capacity of the supplied column is 5 µg. RNA can be eluted into ≥6 µl RNase-free water allowing for a highly concentrated sample.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at -70°C. The addition of RNase inhibitors is recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

**Notes:**

[1] Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate, before use.

[2] For paraffin-embedded tissue sections, use the **Pinpoint Slide RNA Isolation System II™** (R1007).

## Product Description

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

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 *Fast-Spin* column technology to yield high quality purified RNA. This product makes targeted tissue RNA isolation simple and quick. The method does not use any organic solvents or other toxic reagents. There is also no need for expensive specialized equipment. This Pinpoint™ Slide RNA Isolation System I allows for the efficient recovery of RNA from fresh tissue sections for subsequent RNA analyses including RT-PCR.

As outlined below *(Fig. 1)*, simply apply the **Pinpoint™ Solution** to a selected area of tissue on a glass slide. The solution will air-dry forming a thin film that embeds the tissue underneath. The embedded tissue is then lifted from the slide and transferred to a tube. Following treatment of the tissue with **RNA Extraction Buffer**, the extracted RNA is bound, washed and concentrated using the **Zymo-Spin™ IC Column**. The isolated RNA can then be used for analysis including RT-PCR *(Fig. 2)*.

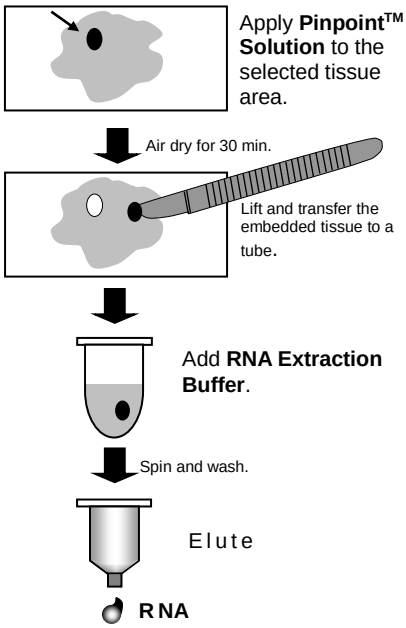


**Figure 1**. Pinpoint System procedure.

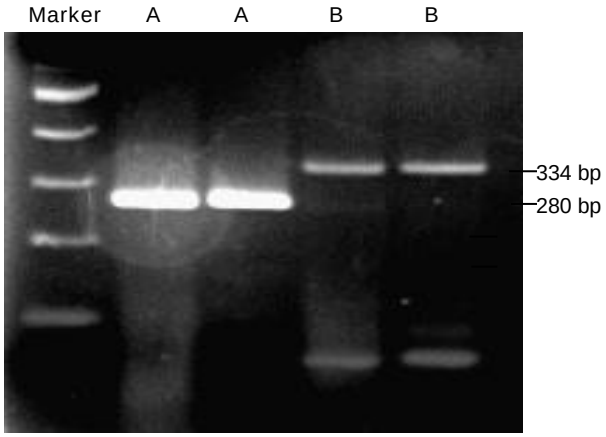


**Figure 2. RT-PCR of human tissue section RNA recovered from tissue using the Pinpoint™ RNA Isolation System I.** Duplicate samples are PCR products from A) human β-actin transcript B) an arbitrary human chromosome 3 transcript.

The **Pinpoint™ Slide RNA Isolation System I** works best with fresh or frozen tissue sections that are fixed by ethanol, acetone, methanol, etc. Although RNA can also be recovered from paraffin-embedded tissues, we found that recovery of RNA from such samples was much less efficient than from fresh tissue sections. This was especially the case when performing RT-PCR of relatively large fragments of cDNA (over 500 bp) as well as of low copy-number mRNAs. The entire Pinpoint procedure includes 3 parts:
**1) Preparation of Tissue Section**, **2) Pinpoint™ Fractionation** to recover tissue from a glass slide, and **3) RNA Extraction** for total RNA recovery.

Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

## Buffer Preparation

Before starting, add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

## Protocol

### I. Preparation of Tissue Sections

1. Use a sterile ethanol/water solution to clean glass sample slides and then dry by autoclaving or baking at 300°C for 4 hours.

2. Mount a tissue section (≥10 µm thick) onto a glass slide and dry it at 60°C for 30 minutes.

3. Submerge the slide in 95% ethanol at room temperature for 60 minutes to fix the section.

4. Air dry the sample on the slide for approximately 30 minutes. RNA isolation can now be performed using the **Pinpoint™ Slide RNA Isolation System I**.

### II. Pinpoint™ Fractionation

(Procedure for the removal of a selected area of tissue from a glass slide.)

1. Apply the **Pinpoint™ Solution**[1] to the area of tissue on the slide where the RNA is to be extracted[2].
   Use a sterile pipette tip or a syringe to gently spread a small amount of the **Pinpoint™ Solution** over the selected tissue region. Generally, use about 0.5 µl of the **Pinpoint™ Solution** per $mm^2$ of tissue area.

2. Allow the **Pinpoint™ Solution** to dry completely at room temperature. (Usually about 30 to 45 minutes).
   The **Pinpoint™ Solution** should dry as a blue film embedding the tissue and cells underneath.

3. Remove the embedded tissue from the slide.
   Use a sterile blade or scalpel to cut then remove the embedded section from the slide. Transfer the sample to an RNase-free tube.

4. Centrifuge briefly to locate the tissue sample to the bottom of the tube.

### III. RNA Extraction

(Procedure for the extraction and purification of total RNA from a tissue sample.)

1. Add 200 µl of **RNA Extraction Buffer** to the tube containing the embedded tissue sample.

2. Lyse the embedded tissue sample by pipetting the **RNA Extraction Buffer** up and down. Vortex briefly.

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

**Notes:**

[1] The **Pinpoint™ Solution** is thick and should be spread using a small implement like a pipet tip.

[2] Normally, a minimum 1 $mm^2$ fresh tissue of 10 µm thickness (approx. 500-1000 cells depending on the tissue type and cell density) is required to achieve adequate RT-PCR results. The area covered by each tissue sample can vary from 1 to 100 $mm^2$ according to the requirements of the researcher.

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

5. Transfer the mixture to the the **Zymo-Spin™ IC Column** in a **Collection Tube**.

6. Centrifuge column at ≥10,000 x *g* in a microcentrifuge for 1 minute. Discard the flow-through.

7. Add 200 µl **RNA Wash Buffer** to the column and centrifuge at ≥10,000 x *g* for 1 minute. Discard the flow-through. Repeat wash step.

8. Transfer the column to a new RNase-free tube.

   Add 10 µl **DNase/RNase-Free Water**[1] directly to the column matrix. Wait for 1 minute then centrifuge at ≥10,000 x *g* for 1 minute and collect the eluted RNA. The RNA can be used immediately or stored at -70 °C.

## Troubleshooting

1. 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.

2. 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 $mm^2$ to over 100 $mm^2$. We recommend that the sample thickness be ≥10 µm.

3. RT-PCR Parameters are not Optimized
   It is recommended that the conditions used for RT-PCR 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.

4. DNA Contamination
   Traces of fragmented DNA may be present in the eluted RNA fraction. DNA-free RNA can be achieved with subsequent DNase I treatment.

**Note:**

[1] To maximize RNA yield, increase the elution volume and/or repeat the elution.

## Ordering Information

| Product Description | Catalog No. | Kit Size |
|---|---|---|
| **Pinpoint™ Slide RNA Isolation System I** | R1003 | 50 Preps. |

| For Individual Sale | Catalog No. | Amount |
|---|---|---|
| **Pinpoint™ Solution** | D3001-1 | 1 ml |
| **RNA Extraction Buffer** | R1003-2-3<br>R1003-2-12<br>R1003-2-50<br>R1003-2-100 | 3 ml<br>12 ml<br>50 ml<br>100 ml |
| **RNA Wash Buffer** (concentrate) | R1003-3-6<br>R1003-3-12<br>R1003-3-24<br>R1003-3-48 | 6 ml<br>12 ml<br>24 ml<br>48 ml |
| **DNase/RNase-Free Water** | W1001-1<br>W1001-4<br>W1001-6<br>W1001-10 | 1 ml<br>4 ml<br>6 ml<br>10 ml |
| **Zymo-Spin™ IC Columns** | C1004-50<br>C1004-250 | 50<br>250 |
| **Collection Tubes** | C1001-50<br>C1001-500<br>C1001-1000 | 50<br>500<br>1000 |

## Related Products

| Product | Description | Prep/Format | Catalog |
|---|---|---|---|
| | **Total RNA Purification** | | |
| **ZR Whole-Blood RNA MiniPrep™** | whole blood, partitioned blood | 50/column<br>100/column | **R1020**<br>**R1021** |
| **ZR-96 Whole-Blood RNA Kit™** | | 2x96/plate | **R1022** |
| **ZR Viral RNA Kit™** | plasma, serum, liquids, cells, tissue | 50/column<br>200/column | **R1034**<br>**R1035** |
| **ZR-96 Viral RNA Kit™** | | 2x96/plate<br>4x96/plate | **R1040**<br>**R1041** |
| **ZR Urine RNA Isolation Kit™** | urine, liquid samples | 50/column | **R1039** |
| ***Quick-RNA*™ MicroPrep** | cells, tissue, buccal cells, buffy coat, plasma, serum, biological liquids | 50/column | **R1050** |
| ***Quick-RNA*™ MiniPrep** | | 50/column<br>200/column | **R1054**<br>**R1055** |
| ***Quick-RNA*™ MidiPrep** | | 25/column | **R1056** |
| **ZR-96 *Quick-RNA*™** | | 2x96/plate<br>4x96/plate | **R1052**<br>**R1053** |
| **ZR RNA MicroPrep™** | cells, tissue, buccal cells, buffy coat, plasma, serum, biological liquids; DNA removal column, small-RNA recovery (≥17nt), *in-column* DNase treatment protocol | 50/column<br>200/column | **R1060**<br>**R1061** |
| **ZR RNA MiniPrep™** | | 50/column<br>200/column | **R1064**<br>**R1065** |
| **Pinpoint™ Slide RNA Isolation System Kit I** | fresh tissue sections | 50/column | **R1003** |
| **Pinpoint™ Slide RNA Isolation System Kit II** | paraffin-embedded tissue | 50/column | **R1007** |
| **ZR Fungal/Bacterial RNA MicroPrep™** | bacteria, yeast, fungi; BashingBead™ lysis | 50/column | **R2010** |
| **ZR Fungal/Bacterial RNA MiniPrep™** | | 50/column | **R2014** |
| **ZR Plant RNA MiniPrep™** | plant parts and tissues; BashingBead™ lysis, RT/PCR inhibitor removal | 50/column | **R2024** |
| **ZR Tissue & Insect RNA MicroPrep™** | insect, small tissue samples; BashingBead™ lysis | 50/column | **R2030** |
| **YeaStar RNA Kit™** | yeast, fungi | 40/column | **R1002** |
| | **RNA Clean-up, Concentration & Recovery** | | |
| **RNA Clean & Concentrator™-5** | modified/labeled/impure/diluted RNA; small-RNA recovery (≥17nt); *acid phenol* extracted RNA directly from aqueous phase, *in-column* DNase treatment protocol | 50/column<br>200/column | **R1015**<br>**R1016** |
| **RNA Clean & Concentrator™-25** | | 50/column<br>100/column | **R1017**<br>**R1018** |
| **RNA Clean & Concentrator™-100** | | 25/column | **R1019** |
| **ZR-96 RNA Clean & Concentrator™** | | 2x96/plate | **R1080** |
| **DNA-Free RNA Kit™** | DNase I treatment; small-RNA recovery (≥17nt) | 50/column<br>200/column | **R1013**<br>**R1014** |
| **Zymoclean™ Gel RNA Recovery Kit** | agarose gel separated RNA | 50/column | **R1011** |
| **ZR small-RNA™ PAGE Recovery Kit** | polyacrylamide gel separated RNA; small-RNA recovery (≥17nt) | 20/column | **R1070** |
| | **DNA/RNA Parallel Purification** | | |
| **ZR-*Duet*™ DNA/RNA MiniPrep** | cells, tissue, liquids; DNA/RNA separation, small-RNA recovery (≥17nt), *in-column* DNase treatment protocol | 50/column | **D7001** |
| | **DNA/RNA Co-Purification** | | |
| **ZR Viral DNA/RNA Kit** | plasma, serum, liquids, cells, tissue | 25/column<br>100/columns | **D7020**<br>**D7021** |