ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Pinpoint™ Slide RNA Isolation System II

Catalog No. R1007

Contents

For Laboratory Use Only

ZYMO RESEARCH CORP.
Toll Free: 1-888-882-9682 • Fax: 1-714-288-9643 • Web: www.zymoresearch.com • E-mail: info@zymoresearch.com

I. General Information

Description

The **Pinpoint™ Slide RNA Isolation System II** is an innovative product designed by those at Zymo Research to isolate RNA from any targeted area of paraffin-embedded tissue on a microscopic slide. 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 RNA. Unlike current UV-based methods, this product makes isolation of paraffin-embedded tissue RNA simple and quick. There is also no need for expensive specialized equipment or computer software. This kit allows for the efficient recovery of RNA from fresh tissue sections for subsequent RNA analyses including RT-PCR.

Note: For freshly prepared tissue sections, use the **Pinpoint Slide RNA Isolation System I™** product from Zymo Research (Cat. No. R1003).

Highlights

- Allows for the isolation of total RNA from paraffin-embedded tissue 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.

Specifications

- **RNA Purity** – Generally, traces of DNA are present in the eluted RNA fraction. DNA-free RNA can be obtained with subsequent use of Zymo Research's **DNA-Free RNA Kit™** (R1013, R1014, R1027, & R1028).
- **RNA Recovery** – Typically, RNA is eluted into as little as 6-10 µl elution buffer allowing for a highly concentrated sample. The RNA binding capacity of the columns is 5 µg.
- **RNA Storage** – Recommended that 1 U/10 µl RNase inhibitor be added to the RNA prior to storage at -70°C.
- **Sample Sources** – Cells from paraffin-embedded tissue sections on glass slides.
- **Stability of Product Reagents** – Integrity of kit components is guaranteed for up to one year from date of purchase.
- **Quality Control** – Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

Note: 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 II	R1007 Amount	Storage Temperature
Pinpoint™ Solution	1 Tube	Room Temp.
Proteinase K & Storage Buffer*	1 set	-20°C
RNA Digestion Buffer	1.2 ml	Room Temp.
RNA Extraction Buffer	3 ml	Room Temp.
RNA Wash Buffer**	6 ml	Room Temp.
RNA Elution Buffer	1 ml	Room Temp.
Zymo-Spin I™ Columns	50 ct.	Room Temp.
Collection Tubes	50 ct.	Room Temp.
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* Add 260 µl Storage Buffer to **Proteinase K** tube prior to first use.
** Ethanol must be added prior to use as indicated on **RNA Wash Buffer** label.

Ordering Information

Product Description	Catalog No.	Kit Size
Pinpoint™ Slide RNA Isolation System II	R1007	50 preps.

For Individual Sale	Catalog No.	Amount
Pinpoint™ Solution	D3001-1	1 Tube
Proteinase K & Storage Buffer	D3001-2	1 set
RNA Digestion Buffer	R1007-1	1.2 ml
RNA Extraction Buffer	R1003-2-3 R1003-2-12 R1003-2-50 R1003-2-100	3 ml 12 ml 50 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-2.4 R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	2.4 ml 6 ml 12 ml 24 ml 48 ml
RNA Elution Buffer	R1003-4-1	1 ml
Zymo-Spin I™ Columns (uncapped)	C1003-50 C1003-250	50 ct. 250 ct.

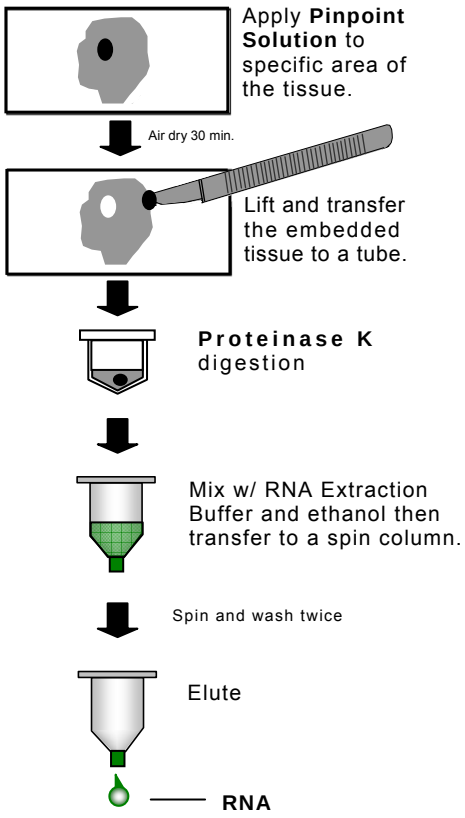
For Individual Sale Cont.	Catalog No.	Amount
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 ct. 500 ct. 1,000 ct.

For **Technical Assistance** contact those at **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

II. Protocol

Overview

As outlined below, 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. This is then lifted from the slide and transferred to a tube. Following treatment of the tissue with **Proteinase K** and **RNA Digestion** and **Extraction Buffers**, the extracted RNA is washed and concentrated using a **Zymo-Spin I™ Column**. The isolated RNA can then be used for subsequent analysis including RT-PCR.



The **Pinpoint™ Slide RNA Isolation System II** is designed for extracting RNA from paraffin-embedded tissue. Thus, paraffin needs to be removed from the tissue prior to beginning the procedure. The entire Pinpoint procedure includes 3 parts: **1) Paraffin Removal from the Tissue Sample**, **2) Pinpoint Fractionation** to recover tissue from a glass slide, and **3) RNA Extraction** for total RNA recovery.

Note: For freshly prepared tissue sections, use the **Pinpoint Slide RNA Isolation System I™** product from Zymo Research (Cat. No. R1003).

Reagent Preparation

- Before starting, add 260 µl **Storage Buffer** to **Proteinase K** tube prior to first use. Also, add 24 ml 100% ethanol to the 6 ml **RNA Wash Buffer** concentrate to obtain the final **RNA Wash Buffer** solution.
- Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

Note: Alternatively, add 26 ml 95% ethanol to the 6 ml size of the wash buffer concentrate.

Method

I. Paraffin Removal from the Tissue Sample

1. Mount the paraffin-embedded tissue section with at least 10 µm thickness onto a glass slide and dry it at 60°C for 30 minutes.

2. Submerge the slide in xylene at room temperature for 1 hour changing the xylene once after 30 minutes.

3. Hydrate the sample by washing progressively for 2 minutes in 100%, 70%, 50% ethanol, and then pure water.

4. Air-dry the sample on the slide. RNA isolation using the **Pinpoint™ Slide RNA Isolation System II™** can now be performed.

II. Pinpoint Fractionation

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

1. Apply the **Pinpoint™ Solution** to the area of tissue on the slide where the RNA is to be extracted.
   -Use a sterile pipette tip or a syringe to gently spread a small amount of **Pinpoint™ Solution** over the selected tissue region. Generally, use about 0.5 µl of **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 a 1.5 ml tube.

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

Note: The **Pinpoint™ Solution** is thick and should be spread using a small implement like a pipet tip.

Note: 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.

III. RNA Extraction

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

1. Add 20 µl of **RNA Digestion Buffer** and 5 µl **Proteinase K** to the tube containing the recovered tissue. Mix gently.
   -For multiple samples, **the RNA Digestion Buffer** and **Proteinase K** may be premixed. Add 25 µl of this mixture to each sample.

2. Incubate the tubes at 55°C for 4 hours.

3. Centrifuge the tubes briefly when the incubation is finished.
4. Add 50 µl (2 volumes) of **RNA Extraction Buffer** and mix.
5. Add 75 µl (1 volume) of 95-100% ethanol to the tube. Lightly vortex.
6. Transfer the mixture to a **Zymo-Spin I™ Column** in a 2 ml Collection Tube.
7. Spin the column at ≥10,000 rpm in a microcentrifuge for 1 minute.
8. Add 200 µl **RNA Wash Buffer** to the **Zymo-Spin I™ Column** and centrifuge at ≥10,000 rpm for 1minute. Discard flow-through. Repeat this step.
9. Transfer the column into a new RNase-free 1.5 ml tube.
10. Add 10 µl of prewarmed **RNA Elution Buffe**r (60°C) directly to the membrane of **Zymo-Spin I™ Column**. Wait for 2 minutes. Spin at ≥10,000 rpm for 1 minute to elute the RNA. [The isolated RNA (2-8 µl) can be used directly for RT-PCR amplification (20-50 µl final volume) or can be stored at -70°C for future use].

Note: If RNA will be quantitated using a spectrophotometer (Abs. 260-280 nm), use RNase-free water to elute RNA from the column.

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 or 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 II**. 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 obtained with subsequent DNase I treatment.

References

1 **Weizsäcker, F. V., Labeit, S., Koch, H. K., Oehlert, W., Gerok, W., and Blum, H. E.** 1991. A simple and rapid method for the detection of RNA in formalin-fixed, paraffin-embedded tissue by PCR amplification. Biochem Biophys Res Commun *174*:176-180.

2 **Greer, C.E., J.K. Lund and M. Manos**. 1991. PCR amplification from paraffin-embedded tissues: recommendations on fixatives for long-term storage and prospective studies. PCR Methods Appl. *1*:46-50.

Popular RNA Purification & Isolation Products from Zymo

Product	Description	Kit Size	Cat No. (Column Format)
Pinpoint Slide RNA Isolation System I™	Isolation of total RNA from targeted tissue areas on a microscope slide of fresh or frozen tissue sections. Perfect for RNA isolation from clinical tissue samples.	50 Preps	**R1003**
Pinpoint Slide RNA Isolation System II™	Isolation of total RNA from targeted tissue areas on a microscope slide of paraffin embedded tissue sections. Perfect for RNA isolation from clinical tissue samples.	50 Preps	**R1007**
Mini RNA Isolation I Kit™	Isolation of trace amounts of RNA from $1x10^1$ to $1x10^5$ cells. Available in both capped and uncapped column formats.	50 Preps 50 Preps	**R1005** (Uncapped) **R1006** (Capped)
Mini RNA Isolation II Kit™	Isolation of RNA from 10^2 to $5x10^6$ cells. Available in both capped and uncapped column formats.	50 Preps 200 Preps 50 Preps 200 Preps	**R1030** (Capped) **R1031** (Capped) **R1032** (Uncapped) **R1033** (Uncapped)
ZR Whole Blood Total RNA Kit™	Isolation of RNA from whole blood samples in 15 minutes.	50 Preps 100 Preps	**R1020** **R1021**
ZR Viral RNA Kit™	Isolation of viral RNA from cell-free body fluids or sample mixtures containing cells at a concentration less than 10^5 cells per ml.	50 Preps 200 Preps	**R1034** **R1035**
ZR-96 Viral RNA Kit™	High-output isolation of viral RNA from cell-free body fluids or sample mixtures containing cells at a concentration less than 10^5 cells per ml.	2x96 Preps 4x96 Preps	**R1040** **R1041**
Urine RNA Kit™	Isolation of total RNA from urine sediment samples. The system employs a unique urine filter to collect cells via a simple syringe “push-through” method.	20 Preps 50 Preps	**R1038** **R1039**
RNA Clean-up Kit-5™	Clean and concentrate 5 µg RNA from any reaction in 2 minutes. 8 µl minimal elution volume. Available in capped and uncapped column format.	50 Preps 200 Preps 50 Preps 200 Preps	**R1015** (Capped) **R1016** (Capped) **R1023** (Uncapped) **R1024** (Uncapped)
RNA Clean-up Kit-25™	Clean and concentrate up to 25 µg RNA from any reaction in 2 minutes. 50 µl minimal elution volume. Available in capped and uncapped column format.	50 Preps 200 Preps 50 Preps 200 Preps	**R1017** (Capped) **R1018** (Capped) **R1025** (Uncapped) **R1026** (Uncapped)
DNA Free RNA Kit™	Efficiently removes DNA from RNA preparations for RT-PCR reactions in 15 minutes. Available in capped and uncapped column format.	50 Preps 200 Preps 50 Preps 200 Preps	**R1013** (Capped) **R1014** (Capped) **R1027** (Uncapped) **R1028** (Uncapped)
Zymoclean Gel RNA Recovery Kit™	Isolation of RNA from agarose gels in 15 minutes. 8 µl minimal elution volume for maximum concentration of RNA.	50 Preps	**R1011**
YeaStar RNA Kit™	Isolation of RNA from a broad spectrum of fungi susceptible to yeast lytic enzyme lysis.	40 Preps	**R1001**

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are chaotropic and are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.