ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Pinpoint™ Slide RNA Isolation System II

Catalog No. **R1007**

Highlights

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

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 II (Kit Size)	**R1007** (50 Preps.)	**Storage Temperature**
Pinpoint™ Solution	1 ml	Room Temp.
Proteinase K[1] (with Storage Buffer)	1 set	-20 °C
RNA Digestion Buffer	1.2 ml	Room Temp.
RNA Extraction Buffer	3 ml	Room Temp.
RNA Wash Buffer[2] (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 paraffin-embedded tissue sections on glass slides.
- **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 260 µl Storage Buffer to **Proteinase K** tube prior to first use.

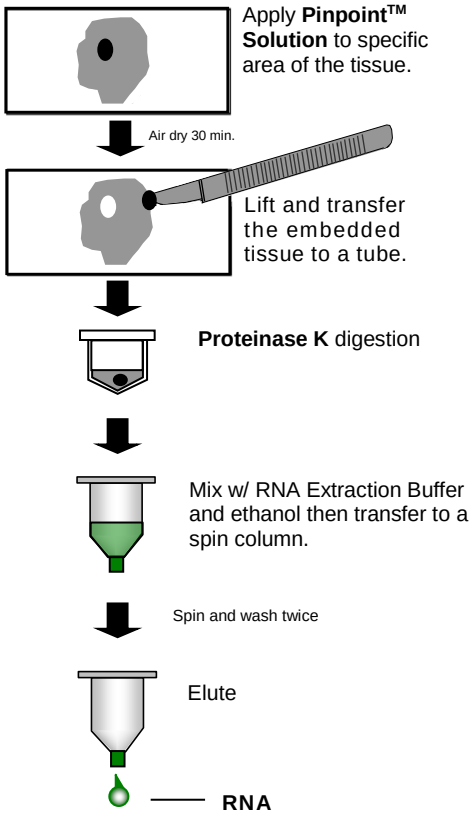
[2] Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate, before use.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended 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.

Product Description

The **Pinpoint™ Slide RNA Isolation System II** is an innovative product designed 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 UV-based methods, this product makes isolation of paraffin-embedded tissue RNA simple and quick. There is also no need for expensive specialized equipment.

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™ IC 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.

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

Note:

For freshly prepared tissue sections, use the **Pinpoint™ Slide RNA Isolation System I** product from Zymo Research (R1003).

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

Buffer Preparation

Before starting, add 260 µl **Storage Buffer** to **Proteinase K** tube prior to first use. Also, add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

Protocol

I. Paraffin Removal from the Tissue Sample

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 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**[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 **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, and 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.

III. RNA Extraction

(Procedure for the extraction and purification of total RNA from a deparaffinized 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.

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.

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 the **Zymo-Spin™ IC Column** in a **Collection Tube**.
7. Spin the column at ≥10,000 x *g* for 1 minute.
8. Add 200 µl **RNA Wash Buffer** to the **Zymo-Spin™ IC Column** and centrifuge at ≥10,000 x *g* for 1minute. Discard flow-through. Repeat this step.
9. Transfer the column into a new RNase-Free Tube.
10. Add 10 µl of prewarmed **DNase/RNase-Free Water** (60°C) directly to the column matrix. Wait for 2 minutes 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 ºC.

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.

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 II	R1007	50 Preps.

For Individual Sale	Catalog No.	Amount
Pinpoint™ Solution	D3001-1	1 ml
Proteinase K (with Storage Buffer)	D3001-2-5	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)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

Related Products

Product	Description	Prep/Format	Catalog
	Total RNA Purification		
ZR Whole-Blood RNA MiniPrep™	whole blood, partitioned blood	50/column 100/column	**R1020** **R1021**
ZR-96 Whole-Blood RNA Kit™		2x96/plate	**R1022**
ZR Viral RNA Kit™	plasma, serum, liquids, cells, tissue	50/column 200/column	**R1034** **R1035**
ZR-96 Viral RNA Kit™		2x96/plate 4x96/plate	**R1040** **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 200/column	**R1054** **R1055**
***Quick-RNA*™ MidiPrep**		25/column	**R1056**
ZR-96 *Quick-RNA*™		2x96/plate 4x96/plate	**R1052** **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 200/column	**R1060** **R1061**
ZR RNA MiniPrep™		50/column 200/column	**R1064** **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 200/column	**R1015** **R1016**
RNA Clean & Concentrator™-25		50/column 100/column	**R1017** **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 200/column	**R1013** **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 100/columns	**D7020** **D7021**