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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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INSTRUCTIONS

Pinpoint Slide DNA Isolation System ™

Catalog No. **D3001**

Highlights

- Easily isolates genomic DNA in any *targeted* microscopic tissue area on a slide.
- The simple procedure combines Pinpoint tissue sampling technology with a one-step DNA extraction method.
- Efficiently recovers genomic DNA from paraffin archives or fresh tissue sections for PCR analysis.

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ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

ZYMO RESEARCH CORP.

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GENERAL INFORMATION

Kit Contents:

Product		Storage Conditions
1 tube	Pinpoint solution	Room Temperature
1 set	Proteinase K and Storage Buffer	-20°C
2.5 ml	PP Extraction Buffer	Room Temperature
6 ml	Pinpoint Binding Buffer	Room Temperature
2.4 ml	PP Wash Buffer (Concentrated. Add 14 ml of 95-100% ethanol before use.)	Room Temperature
50	Zymo-Spin I columns	Room Temperature
50	Collection tubes	Room Temperature
1	Instruction sheet	

Reagents provided in this kit are designed for 50 sample processes.

Reagents not provided in this kit:

Distilled water	TE Buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
Ethanol (50%, 70%, 95-100%)	Xylene

Ordering Information:

Products	Cat No	Size
Pinpoint Slide DNA Isolation System™ Reagents provided are for 50 sample recoveries.	**D3001**	**1 set**
For Individual Sale:		
Pinpoint Solution	D3001-1	1 tube
Proteinase K and Storage Buffer Set	D3001-2-50	1 set
PP Extraction Buffer	D3001-3	2.5 ml
Pinpoint Binding Buffer	D3001-4	6 ml
PP Wash Buffer	D3001-5	2.4 ml
Zymo-Spin I Columns™	C1003-50	50 columns
	C1003-250	250 columns
Collection Tubes	C1001-50	50 tubes
	C1001-500	500 tubes
	C1001-1,000	1,000 tubes

GENERAL DESCRIPTION

The **Pinpoint Slide DNA Isolation System™** is an innovative product designed by Zymo Research to easily isolate genomic DNA in any targeted microscopic tissue area on a slide. The system combines our powerful **Pinpoint** tissue sampling technology with a one-step DNA extraction method and makes targeted tissue genomic DNA isolation easy and simple. The kit efficiently recovers genomic DNA from paraffin archives or fresh tissue sections for PCR analysis. Unlike the UV-fractionation method, the **Pinpoint** system does not destroy the surroundings of target cells. There is no need for expensive specialized equipment or computer software.

Method Principle: As outlined in ***Figure 2,*** simply apply the **Pinpoint Solution** to a selected microscopic tissue area. The solution will air-dry into a thin film while the underlying cells will become embedded within. The area is lifted and transferred into a tube. After digestion with the one-step **Extraction Buffer** (containing proteinase K) the DNA is ready for PCR analysis.

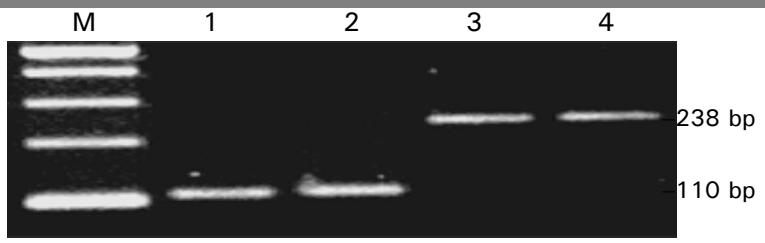

Figure 1. PCR amplified DNA fragments from different samples of paraffin embedded tissue. Lane M: 100 bp marker; Lane 1 and 2: β-Globin; Lane 3 and 4: An uncharacterized gene on chromosome 3.

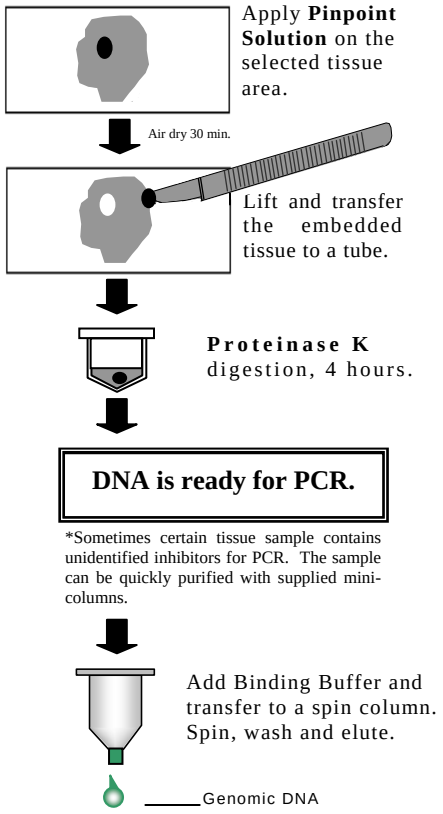

Figure 2. Pinpoint System procedure.

PROTOCOL

Read before starting:

1. Preparation of **Proteinase K** working stock: Simply add 260 µl **Proteinase K Storage Buffer** to the **Proteinase K** tube. Dissolve completely and store at -20°C.
2. Add 14 ml of 95-100% ethanol to the **PP Wash Buffer Concentrate** to make final **PP Wash Buffer**.

Effects of tissue fixing procedure on DNA recovery:

The integrity of DNA from tissue sections is greatly affected by the sample fixing and staining procedures. Different fixing and staining methods cause dramatic effects on the quality of recovered DNA from tissue samples (Refer to references 1 and 2). The major factors that affect the DNA recovery are the fixatives used, duration of fixation, and the age of the sample. Generally, the fixing procedure should be kept under 24 hours for successful recovery of DNA fragments larger than 1 kb by PCR if the tissue is fixed in ethanol, acetone, or OmniFix followed by 10% buffered neutral formalin (BNF). Tissue fixed in highly acidic solution (such as Carnoy's, Zenker's and Bouin's) are usually unsuitable for subsequent DNA amplification because DNA is highly degraded under these conditions. Also, the use of very old samples decreases the success rate of large fragment amplification since DNA is gradually degraded with time. A recent study also showed that different staining procedures are also very important for DNA recovery from tissue sections (Refer to reference 1).

The Pinpoint Slide DNA Isolation System can be used for fresh tissue sections or paraffin archives. For paraffin embedded tissue, the paraffin needs to be removed before starting this procedure, see **Appendix:** "**Deparaffin**" on page 5. For other samples such as frozen sections, tissue sections fixed by acetone, ethanol, methanol, etc., the slides can be used directly by the kit.

Pinpoint Fractionation

The purpose of this procedure is to remove the selected tissue area from the slide.

1. Apply the **Pinpoint Solution** over the area you want to recover DNA on the prepared slide.

   Use a pipette tip or a syringe to gently spread a small amount of **Pinpoint Solution** over the selected region. Use about 0.5 ul of **Pinpoint Solution** per mm^2 of tissue area or cover the selected area with a 0.5 mm layer of **Pinpoint Solution**. It is OK to spread the **Pinpoint Solution** beyond area that you had selected. It is actually easier to define the exact tissue area for recovery in step 3, see below.

2. Dry the **Pinpoint Solution** completely at room temperature. It usually takes about 30 to 45 minutes.

   The **Pinpoint Solution** will change into a thin blue film after completely drying; the underlying tissue cells are embedded in the film at this stage.

3. Remove the embedded tissue from the slide.

   Use a clean and sharp knife to cut the exact area you want, then peel the area off the slide. Transfer the film to a 0.5 ml tube. Generally, a minimal 1 mm^2 tissue area with 10 um thickness (about 200-400 cells depending on different tissue and cell density) is needed for successful gene amplification. The size of each sample spot can vary from 1 to over 20 mm^2 according to your needs.

4. Centrifuge the tubes briefly to bring the fragment to the tube bottom.

References

1 **Burton, M.P., B.G. Schneider, R. Brown, N. Escamilla-Ponce and M.L. Gulley**. 1998. Comparison of histologic stains for use in PCR analysis of microdissected, paraffin-embedded tissues. BioTechniques *24*:86-92.

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.

DNA Extraction

This one buffer extraction procedure will extract DNA from the recovered tissue.

1. Add 50 µl of **Extraction Buffer** and 5 µl **Proteinase K** to the tube containing the recovered tissue. Mix gently.

   For multiple samples, **Extraction Buffer** and **Proteinase K** can be premixed, add 55 µl of this mixture to each sample.

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

3. Heat the tubes at 95°C-98°C for 10 minutes then immediately put them on ice. This can be easily accomplished on a PCR machine.

   Make sure that the temperature is above 95°C. Incomplete inactivation of Proteinase K can cause problems in the PCR reaction.

4. Vortex hard for 10-15 seconds. Centrifuge briefly. The DNA is ready for PCR analysis.

Attention: In most cases the DNA is suitable for PCR analysis at this step and there is no need to proceed to the following **DNA Purification** steps. Use 4-8 µl of above DNA for each PCR reaction in 25-50 µl volume. Sometimes tissue processing procedures, mainly fixing and staining processes, inadvertently add PCR inhibitors to the tissue and the DNA needs further purification to eliminate the inhibitors. Please follow the **DNA Purification** procedure below to purify the DNA further.

DNA Purification

Note: Add 14 ml 95-100% ethanol to the **PP Wash Buffer Concentrate** to make final **PP Wash Buffer**.

1. Add 100 µl **Pinpoint Binding Buffer** to the above **Proteinase K** treated DNA sample. Mix briefly.
2. Transfer the mixture to a **Zymo-Spin I Column** and place the column into a 2 ml **Collection Tube**.
3. Spin the column and tube at full speed in a microfuge for 10 seconds.
4. Add 150 µl **PP Wash Buffer** to the **Zymo-Spin I Column** and centrifuge at full speed for 10 seconds to wash. Add another 150 µl **PP Wash Buffer** and centrifuge at full speed for one minute.
5. Transfer the column into a new 1.5 ml tube.
6. Add 10 µl water or TE Buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) directly to the membrane of **Zymo Spin I Column**. Wait for 1 minute. Spin briefly for 10 seconds to elute the DNA.

The eluted DNA now can be used for PCR amplification or can be stored at -20°C for future uses. Use 2-4 µl of the purified DNA for each PCR reaction in 25-50 µl reaction volume.

APPENDIX

Deparaffin

Preparation of Paraffin sections

1. Mount a tissue section with a 10 µm thickness onto a glass slide and dry it at 60°C for 30 minutes.
2. Drop the slide with paraffin embedded tissue in Xylene at room temperature for 30 minutes, change Xylene and incubate another 30 minutes to remove the paraffin.
3. Hydrate slide through 100%, 70%, 50% ethanol, and sterile water for 2 minutes each.
4. Air-dry the slide and it is ready for DNA isolation using the Pinpoint Slide DNA Isolation System.

TROUBLESHOOTING

A) Following are the most common reasons for no amplification.

1) Probably not enough DNA to amplify.

Make sure you choose the appropriate sample area size in the beginning. Select a tissue sample area of about 1 mm^2 and 10 µm thickness (about 200-400 cells depending on tissue); increase the sampling size if dealing with sections containing fewer cells such as fat tissue and connective tissue. Tissue sampling size can be from 1 mm^2 to over 50 mm^2, depending on your particular situation. We recommend using 4-8 µl of crude or purified DNA solution for each 25 µl of PCR reaction.

2) Proteinase K may have digested Taq polymerase.

Incomplete inactivation of Proteinase K may destroy Taq polymerase. Make sure the digestion tube is heated for 10 minutes above 95°C. Heat-inactivation is highly recommended.

3) PCR parameters are not optimized.

The optimization of PCR parameters mainly depends upon materials and variable primers. A hot start is helpful in remedying nonspecific amplification and increasing the reaction sensitivity in some cases. An increase in annealing and extension time and the number of PCR cycles is recommended for the difficult DNA samples. Use a more sensitive primer labeling method, such as ^{32}P or fluorescence labeling when the PCR product is not evident on agarose gel stained with ethidium bromide.

4) The sample contains PCR inhibitors.

PCR inhibitors could come from either the tissue cells or the fixation and staining processes of the sample. Purify the DNA sample further following the **DNA Purification** procedure on page 4.