



EX-WAX™ DNA Extraction Kit (for Paraffin-Embedded Tissue)

CATALOG NUMBER: S4530 **LOT NUMBER:** PSO1607447

DESCRIPTION: The EX-WAX™ DNA Extraction Kit is intended to extract DNA from paraffin-embedded tissue fixed in 10% Formalin or a non-crosslinking fixative. DNA extracted by this kit is suitable for amplification by PCR*.

LIMITATIONS: The quality of extracted DNA is directly related to the quality of the embedded tissue. Harsh or extended fixation will adversely affect the results.

SUMMARY AND PRINCIPLE: The EX-WAX™ DNA Extraction Kit is qualified on paraffin-embedded mammalian tissues fixed in 10% Formalin or non-crosslinking fixative. DNA is made accessible by protein digestion. DNA is solubilized, while digested proteins are "salted out" and spun to the bottom of the tube. DNA is precipitated, dried under vacuum, and resuspended. Once in solution, the DNA is suitable for amplification by PCR.

MATERIALS SUPPLIED: Sufficient reagents to perform 20 DNA extractions containing three to five 5 µm thick tissue sections per extraction.

Part #	Description	Cap Color	Quantity	Storage
90442	Sterile Distilled Water	Clear	1.25 mL	Room temp.
90443	Digestion Solution	Red	3.0 mL	Room temp.
90444	Extraction Solution	Blue	2.0 mL	Room temp.
90445	Precipitation Solution	White	3.0 mL	Room temp.
90446	Resuspension Solution	Green	2.0 mL	Room temp.
90447	Protein Digesting Enzyme (Powder)	N/A	25 mg	-15 to -25°C

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. Ice cold and room temperature 100% ethanol
2. Micropipettor and tips (1 to 200 µL)
3. Vacuum concentrator system (optional)
4. Microcentrifuge (12,000 rpm)
5. Freezer (-20°C)
6. Water bath at 50°C
7. 1.5 mL microcentrifuge tubes

WARNINGS AND PRECAUTIONS:

1. Wear gloves when isolating and handling DNA to minimize the activity of endogenous nucleases. Use autoclaved pipette tips and 1.5 mL microcentrifuge tubes for additional protection against nucleases.
2. Volumes are optimized for five 5 µm thick sections.
3. Qualified on tissue fixed in 10% Formalin or non-crosslinking fixative.
4. Not recommended for use with fresh, frozen tissues.

PREPARATION OF REAGENTS:

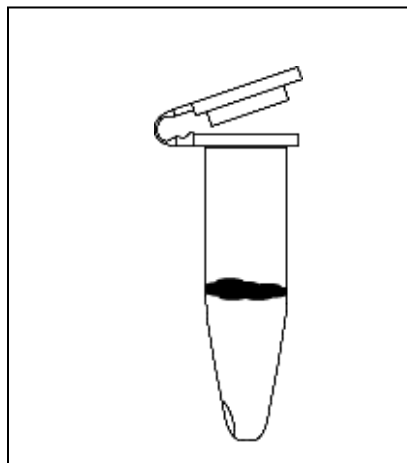
Protein Digesting Enzyme Solution

Add 1.25 mL of Sterile Distilled Water (supplied) to the vial of Protein Digesting Enzyme powder. Mix thoroughly. Store unused solution at -15°C to -25°C.

EXTRACTION PROCEDURE:

1. Cut the tissue sections 5 µm thick. Use 3-5 sections for each extraction.
2. Cut away excess paraffin and place sections in a 1.5 mL tube. Spin briefly to pellet sections.
3. Add 1 mL of fresh 100% ethanol (room temperature) and gently vortex for 15 seconds.
4. Spin for 3 minutes in a microcentrifuge at 12,000 rpm.
5. Remove ethanol and dry pellet in a vacuum concentrator or at 60°C for 10 minutes with the cap open. If residual ethanol is still present, dry for another 10 minutes.
6. Add 150 µL of Digestion Solution and 50 µL of Protein Digesting Enzyme Solution to the tube and mix by stirring. Use the end of the micropipettor tip and physically mix the pellet into the solution. Do not pipette up and down (pellet will not dissolve).
7. Incubate for 4 hours to overnight at 50°C.
8. Add 100 µL of Extraction Solution and mix by inversion (at least 3 times) for 15 seconds. Over-mixing will excessively shear the DNA.
9. Spin in a microcentrifuge for 10 minutes at 12,000 rpm. Remove the supernatant and place it in a fresh 1.5 mL tube. This is accomplished by carefully poking the pipette tip through the paraffin layer on top and withdrawing the supernatant, leaving the paraffin and the pellet behind (see Figure 1). If small amounts of paraffin are removed with the supernatant, they will not affect the extraction.
10. Add 150 µL of Precipitation Solution to the supernatant, invert the tube 3 times, and then add 900 µL of ice cold (-20°C) 100% ethanol. Cap the tube and invert several times to mix.
11. Place at -20°C for at least 1 hour.
12. Spin in a microcentrifuge for 10 minutes at 12,000 rpm. Discard the supernatant.
13. Dry the pellet in a vacuum concentrator or at 60°C with the cap open for 10 minutes. If residual ethanol is still present, dry for another 10 minutes.
14. Add 50 µL of Resuspension Solution and incubate for 1 hour at 50°C in a water bath.
15. Use 1 µL of resuspended DNA for each PCR reaction and run appropriate PCR controls.

Figure 1





Thin layer of
paraffin _____

Supernatant _____

Pellet _____

Troubleshooting

1. When resuspending the DNA in Resuspension Solution, do not heat the DNA higher than 55°C. This will cause degradation of the DNA.
2. If the PCR reaction fails, check for the presence and quality of the DNA as follows: Run 10 µL of extracted sample DNA and molecular weight markers on a 0.8% agarose minigel using a 3 mm (width) toothed comb. Run the bromophenol blue dye front approximately 5 cm into the gel. If no sample DNA is observed, try adding 10-40 µL of sample DNA to the PCR reaction or extract a greater number of tissue sections. If the amount of tissue in the block is small, extract more sections, but if the amount of tissue is very large, you may want to try less.

EX-WAX™ is a trademark of Serologicals Corporation.

** The polymerase chain reaction ("PCR") is covered by one or more of the following U.S. Patents: nos. 4,683,202; 4,683,195; and 4,899,818 issued to Cetus Corporation and owned and licensed by Hoffman-LaRoche Molecular Systems, Inc. Purchase of a Serologicals' PCR-related product does not convey a license to use the PCR process covered by these patents. Purchasers of these products must obtain a license to use the PCR process before performing PCR.*

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