

SinaPure™ DNA- FFPE Tissue

(Kit for the isolation of genomic DNA from formalin-fixed, paraffin-embedded tissues)

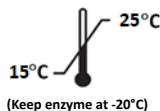
REF

EX6042



50 Tests

RUO



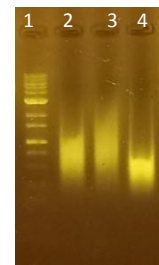
Components

Kit Contents	Quantity/ Volume	Storage Condition
Spin columns	50pcs	RT
PreLysis Buffer	10ml	RT
Proteinase K	1250μl	-20
Lysis Buffer	10ml	RT
Precipitation Buffer	10ml	RT
Wash Buffer I	32.5ml	RT
Wash Buffer II	30ml	RT
Elution Buffer	5ml	RT

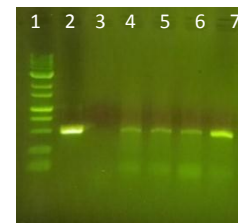
Description

SinaPure™ FFPET is intended to extract DNA from formalin-fixed, paraffin-embedded tissues, FFPET. DNA extracted by this kit is suitable for amplification by PCR.

A combination of detergents, enzyme, reducing digestions, and salts at optimum temperature provide the most suitable condition for selective DNA binding to the silica columns and extraction of DNA from FFPET.



1. DNA Marker
2. Extracted DNA from 25mg deparaffinized thyroid tissue
3. Extracted DNA from 25mg deparaffinized ovarian tissue (250ng/μl)
4. Extracted DNA from 25mg deparaffinized intestinal tissue



1. DNA Marker
2. Positive Control
3. Negative Control
4. PCR Product of 500ng DNA extracted from deparaffinized ovarian tissue
5. PCR Product of 250ng DNA extracted from deparaffinized ovarian tissue
6. PCR Product of 125ng DNA extracted from deparaffinized ovarian tissue
7. PCR Product of 62.5ng DNA extracted from deparaffinized ovarian tissue

Storage and Stability

Spin columns and the kit content are packed in closed bags and can be stored at room temperature (15-25°C). Care should be taken while using, to keep the hygiene state of the columns once opened. Tightly close tubes and bottles immediately after each use.

Proteinase K is delivered as a solution and should be stored at -20°C upon arrival. Following good storage conditions, the performance of the kit content is guaranteed upon the printed expiration date.

Important notes: please read before starting

- The quality of extracted DNA is directly related to the quality of the embedded tissue so please determine quality of the kit with test sample before use.
- Use personal protective equipment and avoid contacting the kit reagents with your eyes and skin.
- When handling human samples, follow recommended procedures for biohazardous materials.
- All buffers are ready to use and no additional alcohol is needed.
- Do not contact the reagents and solutions with acids or bleach solution. It may liberate toxic gas.
- Xylene and 2-Mercaptoethanol needs caution. Avoid inhalation. The fume hood and ventilated area are recommended.
- All centrifugation steps should be done at room temperature (15-25°C).

Not provided equipment and reagents

1	Xylene
2	Ethanol absolute
3	RNase A (Cat. No. MO5411)
4	2-Mercaptoethanol

Preparation of Tissue Sections

Tissue can be digested in 10µm sections or in small portion. Use of thinner sections is awkward because the tissue is very fragile. Thicker sections impede efficient digestion. If more tissue must be sampled to detect rare target sequences, maximum five 10µm sections can be digested in a single tube. Care must be taken to avoid cross-contamination of tissue sections by the microtome blade or the forceps used to handle the paraffin sections. Instruments and blades can be cleaned with xylene to remove residual specimens. If possible, trim excess paraffin from the 10µm sections before placing them in a 1.5ml microcentrifuge tube.

Removal of Paraffin

- 1- Soak the tissue sections in xylene to deparaffinize it. Incubation time depends on the thickness of the section, approx. 30min or more. There after remove the xylene from the sample.
- 2- Add 96-100% ethanol and incubate the tissue sections for 10 sec (Remove the ethanol).
- 3- Add 80% ethanol and incubate the tissue sections for 10 sec (Remove the ethanol).
- 4- Add 60% ethanol and incubate the tissue sections for 10 sec (Remove the ethanol).
- 5- Add 40% ethanol and incubate the tissue sections for 10 sec (Remove the ethanol).
- 6- Incubate the tissue sections in double distilled water (rehydration) for 10 sec (remove the water).

Protocol

Transfer the deparaffinized tissue (25-50mg) to a clean, sterile, pre-weighed 1.5ml micro centrifuge tube. To tissue sample add the following: (Cutting the sample with scalpel in small pieces before incubation can increase the yield of nucleic acids).

1. Add 200µl PreLysis solution to the sample.
2. Add 25µl Proteinase K.
3. Mix the tube immediately and incubate for 1-3 hours at 59°C. It may extend to overnight incubation and then followed by addition of extra 20µl enzyme (Not provided) and 1-3 hours more incubation time.

*Occasionally vortex (every 5-10min). Short vortex during incubation may enhance the final yield.

4. Incubate for 30min at 80°C (can be extended to an hour).
5. If RNA-free genomic DNA is required, add 5µl RNase A (20 mg/ml, not provided) and incubate for 15min at 37°C. Allow the sample to cool to room temperature before adding RNase A.
6. Add 200µl Lysis Buffer and vortex vigorously for 20sec.
*You can add 2-5µl 2-Mercaptoethanol for better digestion (optional).
*In case of remaining insoluble materials briefly spin and transfer the supernatant to a new 1.5ml tube.
7. Add 200µl Precipitation solution. Incubate the tube at room temperature for 3-5 minutes while inverting it occasionally and transfer all tube content to the column quickly (≈650µl).
8. Centrifuge the tube at 12,000rpm for 1 min and discard the filtrate.
9. Add 650µl Wash buffer I to spin column. Centrifuge the tube at 12,000rpm for 1min and discard the filtrate.
10. Add 600µl Wash buffer II to spin column. Centrifuge the tube at 12,000rpm for 1min discard the filtrate.
11. To remove the excess amount of wash buffer II, centrifuge the empty column at 12,000rpm for 3min and discard the collection tube.
12. Carefully transfer the column to a new 1.5ml tube (not included). Place 30-100µl preheated elution buffer in the center of the column, close the lid, and incubate at room temperature for about 5-7 minutes.
13. Centrifuge the tube at 12,000rpm for 1min to elute the DNA.

*The elution volume depends on the sample and may evaluate for your amplification reaction: If high DNA amounts are expected, a higher elution volume (100µl) may increase the DNA yield. Generally, 30-50µl elution volume gives satisfactory results.

*An alternative way of increasing the DNA yield is repeated centrifugation. Transfer 25-90µl of eluted DNA to the center of the membrane filter again. Incubate at room temperature for 3-5 minutes and then centrifuge at 12,000rpm for 1min to increase the DNA yield.

DNA quality control

This kit designed for DNA extraction from FFPE tissues for PCR analysis. In general, agarose gel electrophoresis of prepared DNA is a direct method for testing DNA but, preserved tissues may contain partially degraded DNA. However, fixation times of less than 24h before embedding in paraffin are less likely to result in extensive loss of the DNA template. Depending on the expected amount, pipette 5-10µl eluted DNA directly to a gel slot.

Evaluate and prove amplifiable quality of extracted DNA by amplification of a housekeeping gene. Inhibitors may remove by further purification.

Photometric determination of DNA concentration and quality

Determination of DNA concentration is done by UV reading at 260nm. DNA preparations should be vortexed shortly and diluted (if needed) accordingly by 10mM Tris-HCl or elution buffer. Blank and dilution buffer should be the same. A standard procedure for measuring DNA quality is the determination of the absorption quotient (Q) of readings at A260nm, A280nm, and 230nm. However, these ratios directly depend on the sample quality.

$Q = A_{260nm}/A_{280nm}$. For a pure DNA preparation, Q lies between 1.7 and 2.0. Meanwhile, the A260nm/A230nm ratio represents salt contaminants. This ratio may be placed ≤ 1.5

Kit Quality Control






All components of this Kit are successfully tested in DNA purification from FFPE tissues. Obtained DNA successfully checked in amplification reaction for a 500bp (housekeeping gene, GAPDH) PCR product.

Troubleshooting

This guide may help solve problems that may arise.

Observation	Possible cause	Comments/suggestions
Low or no DNA yield	Inefficient lysis of sample	<ul style="list-style-type: none"> - Make sure that incubation time with enzyme is sufficient. - Short vortex during incubation may enhance the final yield. - Decrease starting materials, extend incubation time and homogenization step.
	Filter may clog during purification	<ul style="list-style-type: none"> - Decrease starting materials. - Check Lysis solution for any crystal formation. - Check lysate for any tissues or particle remaining. - Extend enzymatic incubation time, homogenization step or remove particles. - Before addition of Precipitation Buffer, centrifuge the tube at 12,000rpm for 1min and transfer the supernatant to a new 1.5ml tube.
No enzymatic reaction	Residues of ethanol	<ul style="list-style-type: none"> - Before adding Elution buffer, ensure that the column is dry, and no ethanol contaminates the tip of the column. If you observe residues of ethanol, place the column in a new reaction tube. Centrifuge again for 3 minutes at 12,000rpm.
	PCR Inhibitors	<ul style="list-style-type: none"> - Reduce the template. In general, 0.5- 1μl or less of DNA is sufficient for PCR. - Perform Purification protocol from step one.

Signs

Signs	Definitions	Signs	Definitions
	Temperature range on product use		Name and address of the manufacturer of the product
	For Research Use Only		
	Product shipping conditions		Product technical code




شکایات مشتری

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