CinnaPure DNA- FFPE Tissue

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

Cat. No. PR911683

25 Preparations

Store kit contents at:2-8 °C

Store Ributinase at -20°C

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CinnaPure DNA

Kit for the isolation of genomic DNA from formalin-fixed, paraffin-embedded tissues.
PR911683 25 Preps Store at 2-8˚C
CinnaPure FFPET is intended to extract DNA from paraffin-embedded tissue fixed in 10% Formalin or non-cross linking fixatives. DNA extracted by this kit is suitable for amplification by PCR. 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. In case of any questions please contact **qc@sinaclon.com**

Mini spin columns	25X
Collectiontubes(2.0ML)	25x
Prelysis Buffer	2.5 ml
Ributinase, 100mg/ml	750ul
LysisBuffer	10 ml
PrecipitationBuffer	7.5ml
WashBufferI	10ml
WashBufferII	20ml
ElutionBuffer	2.5ml

Reagent should be supplied by user:

Xylene 100% ethanol 80% ethanol 60% ethanol 40% ethanol Double distilled water (rehydration)

Storage and Stability
Spin columns of the kit are packed in closed bags and show full performance in this state at room temperature (18-25˚C) for at least 1 year. Ributinase is delivered as a solution and should be stored upon arrival at -20 ˚C. This guarantees performance for 1 year.
Please take care that columns, once opened, should be used instantly. Close bottles immediately after use.

Kit Description
This kit contains all ingredients for quick preparation of pure DNA from formalin-fixed, paraffin-embedded tissues, FFPET. The kit contains spin columns, buffers and reagents necessary for lysis of material, DNA binding to the matrix, and washing and elution of DNA into small volume from the matrix. Each kit contains a manual with detailed protocols of DNA extraction.
CinnaPure –FFPET DNA kit system is one of the latest nucleic acid purification technologies. This kit presents remarkable features of timesaving, easy, prompt and high yield DNA purification. Basis of the technology is in brief, tissue section deparaffinized by xylene and then xylene removes by ethanol finally tissue rehydrates in water. Optimal Pre lysis buffer composition during incubation time, enhance tissue degradation and DNA leakage. Appropriate condition of lysis solution binds DNA to matrices including silica membrane filters in presences of high salts concentration and reversibly elution in low salt condition like elution buffer or 10mM Tris-HCl.

This kit supplied with Ributinase or innovative enzyme blend for simultaneously protein and RNA degradation therefore there is no need for further RNase treatment. Obtained DNA is suitable for PCR.
Important notes: please read before starting
Warm Pre lysis and Lysis buffer by placing in 37ºC or more for 15 min and finally softly shake. Heat a water bath or heater block to 55˚C. You need a bench top micro centrifuge (12.100 x g, 13.000 rpm), precision pipettes and sterile pipette tips allowing pipetting volumes 1 to 10 µl, up to 100 µl and up to 1000µl, and sterile 1.5 ml or 2 ml polypropylene tubes. Place spin columns in a rack before starting with the extraction protocol. Open spin columns only directly before use.
All centrifugation steps should be done at room temperature (18-25ºC)

WARNINGS AND PRECAUTIONS

Caution: **Xylene** ,Lysis and wash I Buffer are toxic and irritant. They should be open in fume hood.
Avoid contact any kit reagents with skin & eyes. Wear gloves before use CinnaPure FFPET. Contact of Lysis and wash I buffer Solutions with acids or bleach solution, liberate toxic gas. When handling human samples, follow recommended procedures for biohazardous materials.

Preparation of Tissue Sections

Tissue can be digested in 10/µm 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 10/µm 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 10/µm sections before placing them in a 1.5-ml microcentrifuge tube.

Removal of Paraffin

Note: Xylene is a hazardous chemical. The step 1 must be done in a fume hood and the resulting waste handled appropriately.

1-Soak the tissue section in xylene to deparaffinize it. Incubation time depends on the thickness of the section, approx. 30 min or more.
2- Incubate the tissue section in each of the following for 10 sec:
100% ethanol
80% ethanol
60% ethanol
40% ethanol
Double distilled water (rehydration)
Cut the desired tissue area from rehydrated section with a scalpel. Transfer the sample to a clean, sterile, preweighed 1.5 ml micro centrifuge tube. Determine the weight of the sample. To tissue sample (25-50mg) add the following:
(Cutting the sample with scalpel in small pieces before incubation can increase the yield of nucleic acids)
Prelysis Buffer 100 ul
Ributinase 20 ul
Mix the tube immediately and incubate for:
1-3 hours at 55°C or until complete digestion. It may extend to overnight incubation and then followed by addition of extra 10-20 ul enzyme and 1-3 h more incubation time. Insoluble materials should be removed after lysis step*.
Incubate 10 min at 72°C.

Protocol for Isolation of nucleic acid from formalin-fixed paraffin-embedded tissue or section:

Approximate time for total nucleic acid preparation ≠ **15 min.**

1. Lysis step: Add 400 ul Lysis solution and vortex 20 sec.
*Insoluble materials could be degraded by softly pipetting or removed.
Any remaining material clogs the filter membrane and reduces the final yield significantly.

2. Add 300 µl Precipitation solution and vortex at max speed for 5 seconds.

3 .Transfer the solution to a spin column with collection tube (included) by pipetting.

4 .Centrifuge the tube at (12.100 x g, 13.000 rpm) for 1 min. Discard collection tube.

5. Place spin column in collection tube add 400 µl Wash buffer I to spin column. Centrifuge at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.
6. Wash the spin column with 400 µl of Wash buffer II centrifugation at 12.100 x g (13.000 rpm) for 1 min. Discard flow-through.

7. Wash the spin column with 400 µl of Wash buffer II by centrifugation at 12.100 x g

(13.000 rpm) for 1 min. Discard flow-through.

8. Place spin column in collection tube. Centrifuge at 12.100 x g (13.000 rpm) for 1 min.*
9. Carefully transfer the column to a new 1.5 ml tube (not included). Place 50 µl 65°C pre heated elution buffer in the center of the column, close lid and incubate for 3-5 min at 65°C. Thereafter, centrifuge at 12.100 xg (13.000 rpm) for 1 min to elute the DNA.**

* Avoid contaminating the column with ethanol. 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.

** The elution volume depends on the sample and may evaluate for your amplification reaction: If high DNA amounts are expected, a higher elution volume (100 µl) may increase the DNA yield. Generally, 30-50 µl elution volume gives satisfactory results. An alternative way of increasing the DNA yield is repeated centrifugation. Transfer 25-45 ul eluted DNA to center of membrane filter again and centrifuge at 12.100 xg (13.000 rpm) for 1 min to increase the DNA yield.
This guide may help solve problems that may arise.

Trouble shooting:

Observation	**Possible cause**	**Comments/suggestions**
Low or no DNA yield	Inefficient lysis of sample	Make sure that: 1-incubation time with Ributinase 2-homogenization step by vortex were enough. - Decrease starting materials, extend incubation time and homogenization step.
	Filter may clogged during purification	Check Lysis solution for any crystal formation. Check lysate for any tissues or particle remaining. - Warm lysis before purification - Extend enzymatic incubation time, homogenization step or remove particles. All centrifugation steps should be done at room temperature (18-25°C)
No enzymatic reaction	Residues of ethanol	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 at 12.100 x g (13.000 rpm) for 1 min more**
	PCR Inhibitors	Perform Purification protocol from step one.

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 24 h before embedding in paraffin are less likely to result in extensive loss of the DNA template. Depending on the expected amount, pipette 5-10ul 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.
Due to partially degraded DNA during the fixation process and purification the amplicon size should be less than 300bp.

Photometric determination of DNA concentration and quality:
Determination of DNA concentration is done by UV reading at 260 nm. DNA preparations should be vortexed shortly and diluted (if needed) accordingly by 10 mM Tris-HCl or elution buffer. Blank and dilution buffer should be the same. A standard procedure of measuring DNA quality is the determination of the absorption quotient (Q) of readings at A260nm and A280nm:
Q = A260nm /A280nm. For a pure DNA preparation, Q lies between 1.6 and 2.0.

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 450bp (housekeeping gene,GAPDH) PCR product.