

AGENCOURT® FORMAPURE® KIT

NUCLEIC ACID ISOLATION FROM FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE

Please refer to <http://www.agencourt.com/technical> for updated protocols and refer to MSDS instructions <http://www.beckmancoulter.com/customersupport/msds/msds.asp> when handling or shipping any chemical hazards. For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186.

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Introduction

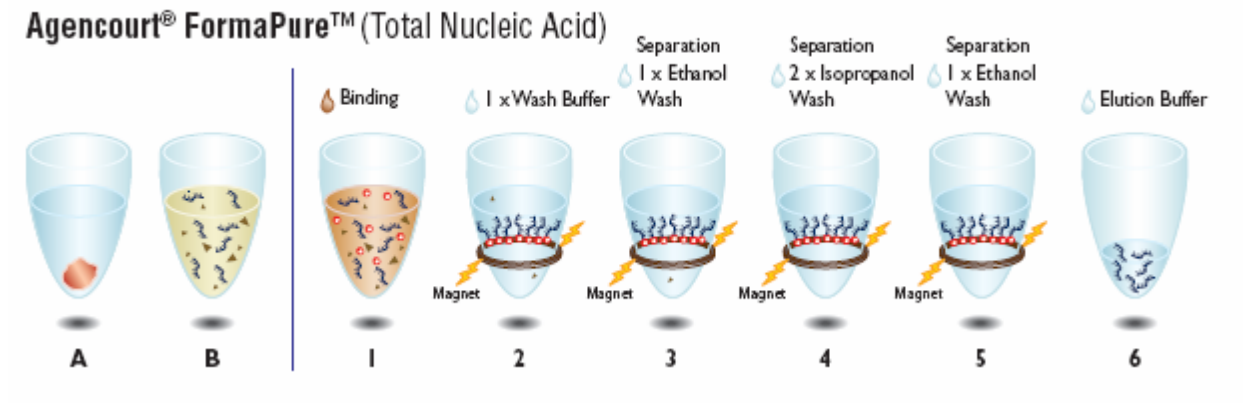
The Agencourt FormaPure nucleic acid purification kit utilizes the patented Agencourt SPRI® paramagnetic bead-based technology to isolate nucleic acids (both DNA & RNA) from a maximum input of 10 mg of formalin-fixed, paraffin-embedded tissue. The protocol can be performed in both 96-well plates (manually and fully automated) and in 1.5 mL tubes. Nucleic acid extraction begins with the addition of a reagent that melts paraffin and de-crosslinks nucleic acids. Proteinase K is then added to complete tissue digestion and inactivate nucleases. Next, binding buffer is added to facilitate immobilization of the nucleic acids to the surface of paramagnetic beads. The contaminants are rinsed away using a simple washing procedure. The Agencourt FormaPure procedure does not require vacuum filtration or centrifugation.



Innovate Automate
SIMPLIFY

Total Nucleic Acid Extraction:

Process Overview



MANUAL

AUTOMATABLE

The Agencourt Formapure procedure for total nucleic acid, is performed in the following stages:

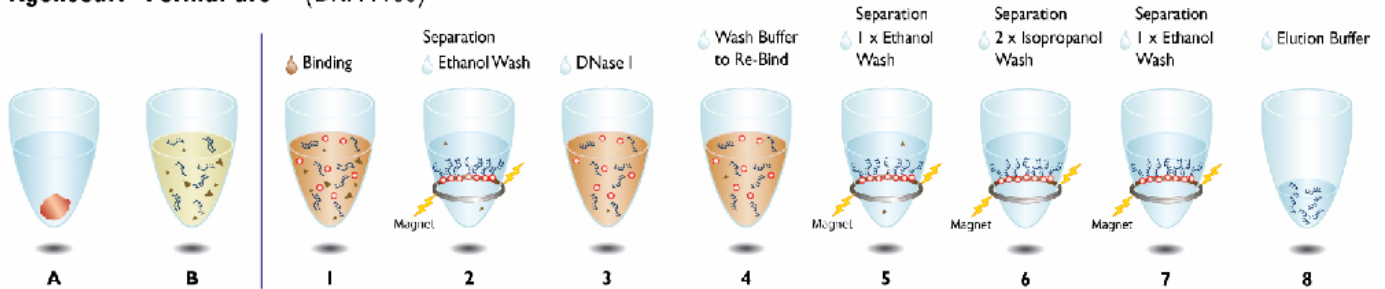
A & B. Digestion of tissue and decrosslinking of nucleic acids

1. Binding of nucleic acids to magnetic beads
2. Separation of beads and washing of captured products with wash buffer
3. Washing with 70% ethanol to remove contaminants
4. Washing with 90% isopropanol
5. Elution of nucleic acids from the magnetic particles

For questions regarding this protocol, call Technical Support at Agencourt 1-800-773-9186

RNA Only:

Agencourt® FormaPure™ (DNA Free)



MANUAL

AUTOMATABLE

The Agencourt FormaPure procedure for the extraction of total RNA, is performed in the following stages:

A & B. Digestion of tissue and decrosslinking of nucleic acids

1. Binding of nucleic acids to magnetic beads
2. Separation of beads and washing of captured products with 70% ethanol
3. DNase I
4. Addition of Wash Buffer to Rebind the RNA
5. Washing with 70% ethanol
6. Washing with 90% isopropanol
7. Washing with 70% ethanol
8. Elution of total RNA from the magnetic particles

Kit Specifications

The Agencourt FormaPure kit is manufactured under RNase-free conditions and has been tested and certified not to contain contaminating nucleases.

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Working Under RNase Free Conditions:

RNases are ubiquitous and general precautions should be followed in order to avoid the introduction of contaminating nucleases during the Agencourt FormaPure procedure. The most common sources of RNase contamination are hands, dust particles, and contaminated laboratory instruments, solutions and glassware. When working with RNA, the following procedures should be followed to limit RNase contamination:

- Always work with gloved hands and change gloves frequently
- Use RNase free, filtered pipette tips for pipetting whenever possible
- Use plastic, disposable consumables that are certified RNase free
- Use dedicated RNase free equipment, e.g. pipettes, pipette tips, gels boxes, etc.
- Avoid using reagents, consumables and equipment that are in common use for other general lab processes
- When available, work in a separate room, fume hood or lab space
- Purchase reagents, such as commonly used buffers and water, that are certified RNase free. Prepare small individual aliquots of such buffers to avoid repeated transfer out of stock buffers. This lowers the risk of contaminating the stock solution
- Wipe down work surfaces with commercial RNase inhibiting surfactant solutions or 70% ethanol before starting work
- Treat electrophoresis gel boxes, including combs and gel trays, with 3% hydrogen peroxide for 10 minutes and rinse with DEPC treated water before use

Materials Supplied in the Kit:

Reagent	Storage Conditions on Arrival	Storage Conditions once In Use
Agencourt FormaPure Lysis Buffer	Room Temperature	Room Temperature
Agencourt FormaPure Bind 1 Buffer	Room Temperature	Room Temperature
Agencourt FormaPure Bind 2 Buffer	4°C	Prepare fresh
Agencourt FormaPure Wash Buffer	Room Temperature	Room Temperature*
PK (Proteinase K)	-20°C	-20°C**
PK buffer	Room Temperature	-20°C***

* with isopropanol

** with PK Buffer

*** with Proteinase K

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Materials Supplied by the User:**Consumables and Hardware:**

- Agencourt Supermagnet magnetic plate (Agencourt product # 000322; <http://www.agencourt.com>) or SPRISand for 1.7 mL tubes (Agencourt product # 001139)
- **For Manual Digestion/Processing 1.2 mL plates** (ABGene Cat # AB1127; <http://www.abgene.com>)
- **For Automated Digestion/Processing 2.2ml Ritter Deepwell plates** (Abgene, Cat# DW 9622; www.abgene.com)
- Adhesive plate film (ABGene Cat # AB-0558)
- 70-72°C and 55°C water bath
- **Optional:** 37°C water bath for DNase incubation.
- Liquid handling robotics or a hand pipette
- For tube format only: 1.7 mL microcentrifuge tubes (ABGene Cat # T6050G; <http://www.abgene.com>)
- Microtome for tissue sectioning

Reagents:

- 100% Isopropanol, ultra pure (American Bioanalytical product # AB-07015; <http://www.americanbio.com/>)
- 90% Isopropanol made from ultra pure isopropanol and nuclease free water
- Fresh 70% ethanol made with nuclease free water (**Note: 70% ethanol is hygroscopic. Fresh 70% ethanol should be prepared for optimal results**) (American Bioanalytical product # AB-00138; <http://www.americanbio.com/>)
- **Optional:** DNase I (RNase-free) [2U/uL]; cat#2222, Ambion Inc., <http://www.ambion.com>]
- **Optional:** DNase I 10X buffer (cat# 8170G, Ambion Inc., <http://www.ambion.com>)
- Reagent grade water, nuclease-free (Ambion product # 9932; <http://www.ambion.com>)

Agencourt FormaPure 96-well & Tube Protocol:

Assembly Steps 1 and 2 are only performed once for each new Agencourt FormaPure kit. If you have already made the following preparations during a previous experiment, please skip ahead to Step 3.

Combine PK Buffer and PK based on the chart below.

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	50 Prep Kit Part # A33341, A33345 and #A33346	96 Prep Kit Part # A33342	384 Prep Kit Part # A33343
Volume of PK Buffer to Add	1.2 mL	2.3 mL	2.3 mL/ tube

For the 50 prep kit, add PK buffer directly to the PK tube. For 96 and 384 prep kits combine buffer and enzyme in a separate tube or bottle. Cap the bottle and mix well by gently shaking the bottle for one minute. Once the solution has been thoroughly mixed, store at -20°C.

1. Add 100% Isopropanol to the Wash Buffer according to the chart below.

	50 Prep Kit	96 Prep Kit	384 Prep Kit
Volume of 100% Isopropanol	15 mL	28 mL	112 mL

2. Invert bottle to mix. Once the solution has been thoroughly mixed, store at room temperature.

3. Prepare Binding Buffer 2 by combining 20 µL Bind 2 with 300 µL of 100% isopropanol for each individual isolation in an RNase free vessel of suitable size (for example: for 10 isolations, add 200 µL of Bind 2 to 3 mL of 100% isopropanol in a 15 mL conical tube).

Vortex Bind 2 thoroughly before combining. Unused solution should be discarded.

4. Pipette 200 µL of Lysis buffer into each well of the 1.2 mL plate to be used (or into the 1.7 mL tube).

5. Prepare DNase solution if you wish to perform a DNase treatment:

Prepare this solution fresh and per isolation – discard any unused solution.

Combine 80 µL nuclease free water, 10 µL 10X DNase buffer, and 10 µL of DNase I.

6. Transfer FFPE tissue sections (up to 5 sections of 10µm each) into the plate/ tube.

For tissue sections attached to glass slides, wet the section with 20 µL of Lysis buffer *prior* to scraping them off with a clean single-edge razor blade. This allows the tissue sections to be more easily transferred to the plate/ tube. Push the tissue section into the plate/tube with a pipette tip. Optimal amount of starting material needs to be scaled according to the size of tissue from 1-5 10µm slices.

7. Seal and incubate the plate/ tube at 70-72°C in a water bath for 60 minutes.

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Prolonged incubation at 70-72°C may cause damage to the RNA. Note: When using this plate in conjunction with a water-bath, make sure the plate does not tip over and the seal does not get wet. Should the seal get wet or condensation form on it, spin the liquid down and very carefully remove the seal.

8. **Following the 70-72°C incubation, pipette 20 µL of PK (40mg/mL from Step1) to each well/ tube, pipette mix twice with a mix volume of 200 µL.**
9. **Seal and incubate the plate/ tube in a water bath at 55°C for 60 minutes.**
10. **Cool the plate on ice for 2 minutes.**
11. **Transfer the lysate to a new 1.2 mL plate/ 1.7 mL tube for nucleic acid extraction.**
12. **Add 150 µL of Bind I Buffer and 320 µL of Bind II Buffer (as prepared in Step 3) to each well/ tube. Pipette mix 5 times with a mix volume of 600 µL. Seal plate with a plate seal and incubate in 55°C water bath for 5 minutes.**
13. **Move the plate onto the Agencourt Supermagnet (or SPRISStand for tubes) and separate for 5 minutes.**

Wait for the solution to clear before proceeding to the next step.

14. **Slowly aspirate the cleared solution from the plate/ tube and discard.**

This step must be performed while the plate/ tube is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

For RNA Only Extraction: Skip to step 18.

It is not necessary to perform the Wash step if you plan to do the DNase treatment.

For Total Nucleic Acid Extraction:

15. **Remove the plate/ tube from the magnet and add 300 µL of Wash buffer. Pipette mix 5 times and incubate for 1 minute.**
16. **Return plate to the magnet and separate for 1 minute.**
Wait for the solution to clear before proceeding to the next step.
17. **Slowly aspirate the cleared solution from the plate/ tube and discard.**

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This step must be performed while the plate/ tube is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

- 18. Remove the plate/ tube from the magnet and add 750 μ L of 70% ethanol. Pipette mix 5 times with a volume of 500 μ L to resuspend the beads.**

- 19. Return plate/ tube to the magnet and separate for 1 minute.**

Wait for the solution to clear before proceeding to the next step.

- 20. Slowly aspirate the cleared solution from the plate/ tube and discard.**

This step must be performed while the plate/ tube is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

For Total Nucleic Acid Extraction: skip to step 30.

For RNA Only Extraction:

- 21. Add 100uL of DNase solution with the plate OFF the magnet.**

- 22. Pipette mix 5 times to resuspend the beads in the DNase solution.**

- 23. Seal and incubate plate/tube in a 37°C water bath for 15 minutes to facilitate digestion of DNA.**

- 24. DO NOT REMOVE THE DNase SOLUTION. Add 550 uL of Wash Buffer and pipette mix 5 times. Incubate at room temperature for 5 minutes.**

- 25. Place plate/tube onto the magnet and separate for 10 minutes.**

Wait for the solution to clear before proceeding to the next step.

- 26. Slowly aspirate the cleared solution from the plate/ tube and discard.**

This step must be performed while the plate/ tube is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

- 27. Remove the plate/tube from the magnet and add 750uL of 70% Ethanol. Pipette mix 5 times.**

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28. Place plate/tube onto the magnet and separate for 5 minutes.

Wait for the solution to clear before proceeding to the next step.

29. Slowly aspirate the cleared solution from the plate/ tube and discard.

This step must be performed while the plate/ tube is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

30. Remove the plate/ tube from the magnet and add 500 μ L of 90% isopropanol. Pipette mix 5 times with a volume of 400 μ L to resuspend the beads.**31. Seal and incubate the plate/ tube in a 70°C water bath for 3 minutes.****32. Return plate/ tube to the magnet and separate for 1 minute.**

Wait for the solution to clear before proceeding to the next step.

33. Aspirate the cleared solution from the plate/ tube and discard.

This step must be performed while the plate is situated on the magnet. Do not disturb the separated magnetic beads.

34. Repeat steps 31-35 for a total of 2 isopropanol washes.**35. Remove the plate from the magnet and add 750 μ L of 70% ethanol. Pipette mix 5 times with a volume of 500 μ L.****36. Return the plate/ tube to the magnet and separate for 1 minute.**

Wait for the solution to clear before proceeding to the next step.

37. Slowly aspirate the cleared solution from the plate and discard.

This step must be performed while the plate/ tube is situated on the magnet. Do not disturb the separated magnetic beads. If beads are drawn out, leave a few microliters of supernatant behind.

38. Let the plate/ tube air dry for 10 minutes.**39. The plate/ tube should air-dry until the last visible traces of ethanol evaporate. Over-drying the sample may result in a lower recovery.**

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- 40. Remove the plate/ tube from the magnet and add 80 µL of nuclease-free H₂O. Resuspend the beads by pipette mixing 5 times.**

Smaller or larger elution volumes can be used for more or less concentrated product, however the minimum elution volume should be 40 µL to ensure complete elution. Optimal elution volumes need to be experimentally determined, higher yielding samples require larger elution volumes due to potential bead carryover during the final transfer.

- 41. Incubate the plate/tube at 65°C -70°C for 30 seconds.**
- 42. Place the plate/tube on the magnet for 1 minute and transfer eluted nucleic acid to a suitable 96 well storage plate or a fresh tube.**

Wait for the solution to clear before transferring sample.

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