# AGENCOURT® DNAdvance™

## Genomic DNA Isolation Kit

*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 DNAdvance™ DNA Isolation Kit utilizes Agencourt's patented SPRI® paramagnetic bead technology to isolate genomic DNA from a variety of sources. This protocol provides instructions to extract DNA from fresh or frozen rodent tails. The protocol is performed in 96-well format. Purification begins by the addition of a lysis buffer, DTT, and Proteinase K to rupture cell membranes and digest protein. DNA is then immobilized on magnetic particles by the addition of a magnetic binding reagent. This differential binding allows the DNA to be easily separated from contaminants using a magnetic field. Contaminants can then be rinsed away using a simple washing procedure, leaving the genomic DNA ready for elution from the magnetic particles. The 96-well plate format procedure is highly amenable to automation since it utilizes magnetic separation, thus eliminating the need for vacuum filtration or centrifugation.

Genomic DNA from the Agencourt DNAdvance™ Kit can be used in:

- Agarose gel analysis
- PCR[1] amplification
- Restriction enzyme digestion
- Membrane hybridizations (e.g., Southern and dot/slot blots).
- AFLP, RFLP, RAPD, microsatellite and SNP analyses (for genotyping, fingerprinting, etc.)

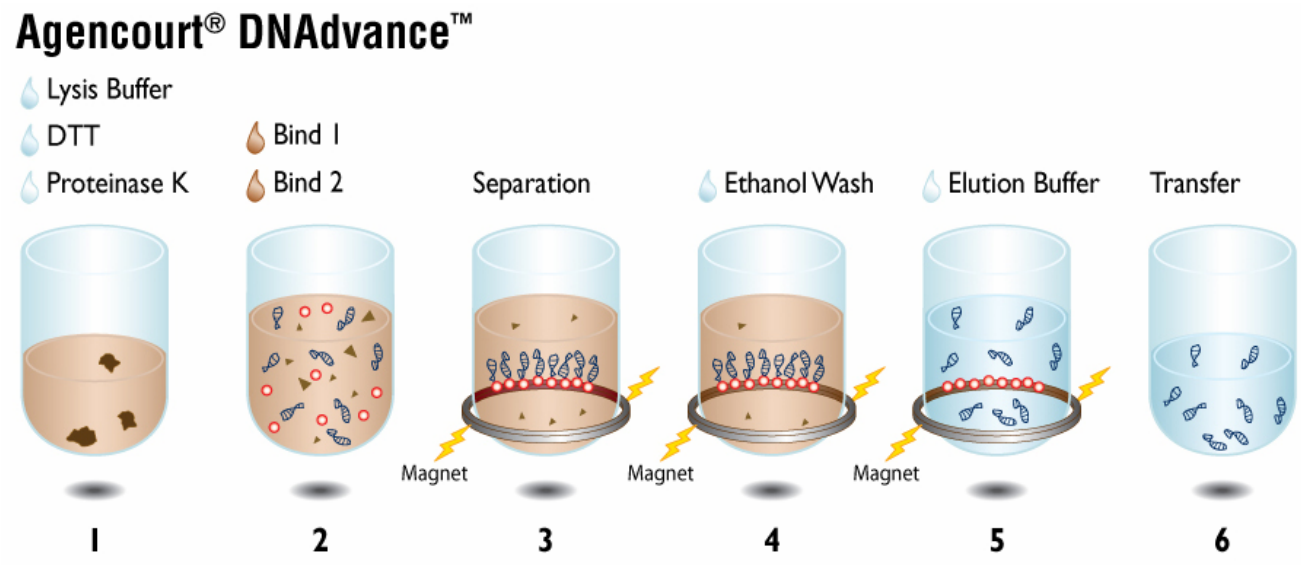
## Warnings and Precautions

1. Agencourt Bioscience Corp. kits are intended ***For Laboratory Use*** only.

[1] *The PCR process is covered by patents owned by Roche Molecular Systems, Inc., and F. Hoffman-La Roche, Ltd.*

2. The U.S. Centers for Disease Control, the Food and Drug Administration, and the American Hospital Association recommend applying "universal precautions" when handling subject's specimens to protect health care and laboratory workers. Under universal precautions, all subjects are considered potentially infectious for blood-borne pathogens. It is recommended that workers protect themselves from contact with the specimens by wearing Proper Protective Equipment which includes gloves, goggles, and lab coats.
3. Please read Material Safety Data Sheet before proceeding the protocol. MSDS can be found at http://www.beckmancoulter.com/customersupport/msds/msds.asp.

**Process Overview:**




1. Lyse up to 20mg specimen in Lysis Buffer, DTT, and Proteinase K.
2. Bind genomic DNA to paramagnetic beads.
3. Separate beads from contaminants.
4. Wash the magnetic beads with 70% Ethanol to remove contaminants.
5. Elute DNA from magnetic particles.
6. Transfer to new plate.

**Kit Specifications:**

| Kit Type | Number of Preps |
|---|---|
| Small Kit A48705 | 384 |
| Large Kit A48706 | 9600 |

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**Reagents supplied in the kit:**

| Reagent | Description | Storage Condition Upon Arrival |
|---|---|---|
| **Lysis Buffer** | Lysis (clear) | Room Temperature |
| **Proteinase K** | Lyophilized Enzyme | -20°C |
| **Proteinase K Buffer** | Proteinase K Buffer (clear) | Room Temperature |
| **Bind1 Buffer** | Binding solution (clear) | Room Temperature |
| **Bind2 Buffer** | Magnetic Solution | 4°C<br>DO NOT FREEZE |
| **Elution Buffer** | 12mM Tris pH 8.0 | Room Temperature |

The reagents have a shelf life of 6 months if stored as directed.

*Required Consumables and Hardware:*

- **Magnetic Separator:**
  For 96 well format: Agencourt SPRIPlate® 96 Ring Super Magnet plate (Agencourt #000322; http://www.agencourt.com/ ; Beckman Coulter A32782 www.beckmancoulter.com/)
- **Consumables:**
  For 96 well format: 96 well 1.2 mL magnet compatible deepwell block (ABGene #AB-1127; http://www.abgene.com)
  96 well 2.2 mL Deepwell block (ABgene/Marsh #DW9622; http://www.abgene.com)
  Pyramid-Bottom Reservoir, Innovative microplates, part number S30014
  Pipettes: p20, p200, and p1000 with aerosol barrier tips
  Seals (for 96 well format): 96 Cap Sealing Mat (ABGene Cat # AB-0662; http://www.abgene.com)
  Incubator Shaker: New Brunswick Scientific, C25; www.nbsc.com, or any shaking incubator that can be set to 55°C and 100 rpm.

*Reagents:*

- 100% Ethanol 200 proof
- 1M DTT (Sigma Aldrich, 43816, http://www.sigmaaldrich.com/).

**Purification Procedure (For up to 20 mg of specimen):**

**Starting Material:** The Agencourt DNAdvance Kit is designed for fresh frozen rodent tails. However, DNA from various tissue types could be extracted with this protocol.

Please note that modifications to this protocol were necessary for automated processing with the Agencourt DNAdvance method for Biomek 96.

**Agencourt strongly recommends using aerosol-barrier (filter) pipette tips when performing the Agencourt DNAdvance purification.**

1. **For each new kit, assemble Proteinase K Solution according to the following chart:**

| Kit | Volume of PK Buffer to add to Proteinase K bottle, final concentration of 40 mg/mL |
|---|---|
| **384 Kit (A48705)** | 3.25 mL |
| **9600 Kit (A48706)** | 81 mL |
| **Storage condition once prepared** | -20°C |

2. **Cut up to 20 mg of sample (equivalent to 10 mm of mouse tail tip) into small pieces and put them into each well of a 1.2 mL plate.**

   Lysis of sample is most effective when samples are cut into small pieces. The samples can be prepped at room temperature.

   For fresh frozen sample from 10 to 20 mg, incubate it at 37°C for 30 minutes before adding the lysis buffer.

3. **Make the following Lysis Master Mix:**

| Component | Volume (µL) |
|---|---|
| Lysis Buffer | 188 |
| 1M DTT | 5 |
| Proteinase K (40mg/mL) | 7 |

4. **Add 200 µL of Lysis Master Mix into each well and seal the plate with a 96-cap sealing mat.**

5. **Incubate the plate at 55°C in a shaking incubator overnight (18-20 hours) at 100 rpm.**

   Shaking while incubating at 55°C helps digestion. To prevent evaporation place a heavy metal block on top of the sealing mat. Please note that for specimen that has less than 10 mg of mouse tail tip, this

incubation time can be shortened to 4 hours following 37°C for 30 minutes prior to adding lysis buffer.

6. **Remove the plate from the water bath or heat incubator.**

7. **Quick spin the plate to remove any condensation before unsealing the plate.**

   Digested lysate can be kept at -80°C if the subsequent steps are not performed immediately**.**

8. **Transfer the lysate into a new 1.2 mL plate.**

9. **Add 100 µL of Bind1 buffer and pipette mix 10 times or until mixed well.**

10. **Shake Bind2 bottle until bead particles are resuspended well in solution.**

11. **Add 170 µL of Bind2 buffer in each well and pipette mix 15 times or until mixed well.**

    During this step, DNA binds to the magnetic particles. When mixing, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles. Air bubbles can trap magnetic beads and prevent them from being pulled to the bottom of the plate, thus decreasing yield.

12. **Incubate the plate at room temperature for 1 minute.**

13. **Place the sample plate on an Agencourt SPRIplate Super magnet for 4 minutes to separate.**

14. **Aspirate and discard the supernatant while the plate is situated on the magnet.**

    When aspirating, place the pipette at the center of the well to avoid disturbing the ring of magnetic beads.

15. **Take the plate off the magnet. Add 340 µL of 70% Ethanol and pipette mix 20 times or until the magnetic beads are resuspended from the bottom of the well.**

    Make fresh 70% Ethanol for each extraction. Pipette mix until the magnetic beads are back in suspension.

16. **Place the plate back on the magnet for 1 minute, or until the solution clears.**

17. **Aspirate and discard the supernatant while the plate is situated on the magnet.**

    Avoid disturbing the ring of magnetic beads.

18. **Repeat steps 15 through 17 twice more for a total of three Ethanol washes.**

19. **Remove as much of the final Ethanol wash as possible before adding Elution Buffer.**

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20. **<u>Take the plate off the magnet.</u> Add 200 µL of Elution Buffer and pipette mix 10 times or until the magnetic beads are completely resuspended from the bottom of the well.**

21. **Place the plate back on the magnet for 5 minutes, or until the solution clears.**

22. **Transfer 190 µL of supernatant to a clean plate or tubes for storage.**

    Aspirate slowly and do not disturb the ring of beads while pipetting. Transferring all 200 µL of product is not recommended as it may carry over some magnetic beads. If beads are being aspirated during the transfer, dispense the sample back into the plate and incubate for another 5 minutes and then aspirate slowly.

23. **Store DNA at -80° for further use.**

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