

Agencourt DNAdvance

Genomic DNA Isolation Kit

Please refer to www.beckmancoulter.com/ifu for updated protocols. For questions regarding this protocol, call Technical Support at Beckman Coulter at 1-800-369-0333.

Agencourt DNAdvance is intended for molecular biology research applications. It is not intended or validated for use in the diagnosis of disease or other conditions.

Introduction



The Agencourt DNAdvance DNA Isolation Kit utilizes Beckman Coulter'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 as it utilizes magnetic separation, eliminating the need for vacuum filtration or centrifugation.

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

- Agarose gel analysis
- PCR 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

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.

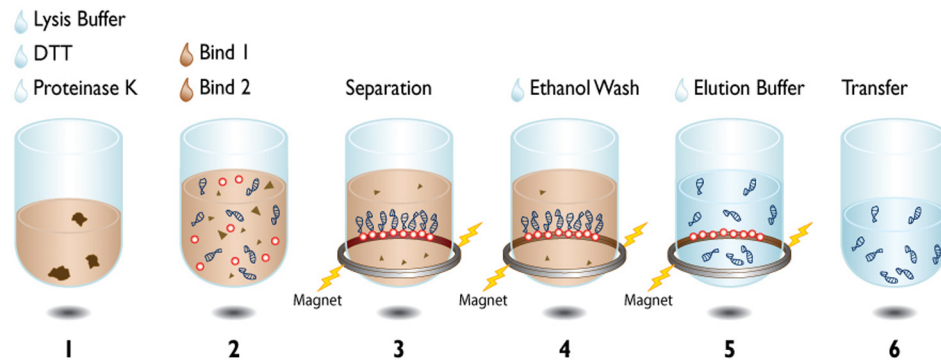
	DANGER
	Proteinase K: Proteinase K 80 – 100%
	H315 Causes skin irritation.
	H319 Causes serious eye irritation.
	H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
	H335 May cause respiratory irritation.
	P261 Avoid breathing vapours.
	P280 Wear protective gloves, protective clothing and eye/face protection.
	P284 In case of inadequate ventilation, wear respiratory protection.
	P304+P340 IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing.
	P312 Call a POISON CENTER or doctor/physician if you feel unwell.
	P403+P233 Store in a well-ventilated place. Keep container tightly closed.
	SDS Safety Data Sheet is available at techdocs.beckmancoulter.com .

WARNING
Bind 1: Thiocyanate Sodium 20 – 30%
H303 May be harmful if swallowed.
H313 May be harmful in contact with skin.
H412 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
P312 Call a POISON CENTER or doctor/physician if you feel unwell.
SDS Safety Data Sheet is available at techdocs.beckmancoulter.com .

WARNING
Lysis: Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate 1 – 5%
H316 Causes mild skin irritation.
P332+P313 If skin irritation occurs: Get medical advice/attention.
SDS Safety Data Sheet is available at techdocs.beckmancoulter.com .

Process Overview

Agencourt DNAdvance



1. Lyse up to 20 mg of 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

Reagents Supplied in the Kit

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

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

Required Consumables and Hardware

- **Magnetic Separator:**

For 96-well format: Agencourt SPRIPlate 96R - Ring Super Magnet Plate
(Beckman Coulter product # A32782 www.beckmancoulter.com/)

- **Consumables**

For 96-well format: 96-well 1.2 mL magnet compatible deepwell block
(Thermo Scientific product # AB-1127)
96 well 2.2 mL Deepwell block (World Wide Medical 99181000)
Pyramid-Bottom Reservoir
(Seahorse Bioscience; product # 201244-100)

Pipettes: p20, p200, and p1000 with aerosol barrier tips

Seals (for 96-well format): 96 Cap Sealing Mat (Thermo Scientific product # AB-0662;
<http://www.fishersci.com>)

Incubator Shaker: 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 sample)

Starting Material: The Agencourt DNAdvance Kit is designed for fresh or 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 the Biomek 96.

Beckman Coulter Inc. 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

- 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 prepared at room temperature.

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

- Make the following Lysis Master Mix:**

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

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

- 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.

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

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

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

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

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

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

- 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.

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

- Place the sample plate on an Agencourt SPRIplate Super magnet for 4 minutes to separate the beads from the solution.**

- 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.

- 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.

- 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 two more times for a total of three Ethanol washes.

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

20. *Take the plate off the magnet. Add 200 μ L 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 μ L 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 μ L 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°C for further use.

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