Instructions For Use

DNAdvance

Genomic DNA Isolation Kit

PN B66866AC
May 2019

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BECKMAN COULTER

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Contact Us

- For questions regarding this protocol, call Technical Support at Beckman Coulter at 1-800-369-0333.
- For additional information, or if damaged product is received, call Beckman Coulter Customer Service at 800-742-2345 (USA or Canada) or contact your local Beckman Coulter Representative.
- Refer to *www.beckman.com/techdocs* for updated protocols.

Glossary of Symbols is available at www.beckman.com/techdocs (PN C05838).

Product Availability

REF C42213 — DNAdvance, 50 Prep Kit

REF A48705 — DNAdvance, 384 Prep Kit

REF A48706 — DNAdvance, 9600 Prep Kit

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Revision History

This document applies to the latest version and higher versions. When a subsequent version changes the information in this document, a new issue will be released to the Beckman Coulter website. For updates, go to www.beckman.com/techdocs and download the latest version of the manual.

Revision AC, 05/2019
Updates include: Format and content updates throughout the manual.

Protocol for Genomic DNA Isolation

DNAdvance *is for molecular biology research use only. Not for use in diagnostic procedures.*

Table of Contents

Product Description

The 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 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.)

Kit Specifications

Kit Part Number	Number of Preps
C42213	50 preps
A48705	384 preps
A48706	9600 preps

Statement of Warnings

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	
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 vapors.
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.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P403+P233	Store in a well-ventilated place. Keep container tightly closed.
SDS	Safety Data Sheet is available at *www.beckman.com/techdocs*.

WARNING	
Pre-Bind PBBA: 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 www.beckman.com/techdocs.

WARNING	
Lysis LBH: 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 www.beckman.com/techdocs.

Storage and Stability

NOTE Refer to the product labels for expiration dates.

Reagent	Storage Condition
Lysis LBH	**Room Temperature**
Pre-Bind PBBA	**Room Temperature**
Bind BBE	**4°C** DO NOT FREEZE
Elution EBA	**Room Temperature**
Proteinase K	**-20°C**
PK Buffer	**Room Temperature**

Materials Supplied

Reagent	50 Preps Kit (C42213)	384 Preps Kit (A48705)	9600 Preps Kit (A48706)	Symbol
Lysis LBH	REF **C42178**	REF **C42197**	REF **C42203**	
Pre-Bind PBBA	REF **C42179**	REF **C42198**	REF **C42204**	
Bind BBE	REF **C42193**	REF **C42199**	REF **C42205**	
Elution EBA	REF **C42195**	REF **C42201**	REF **C42207**	
Proteinase K	REF **C42196**	REF **C42202**	REF **C42208**	–
Proteinase K Buffer (PK Buffer)	REF **C42302**	REF **C42302**	REF **C42151**	–

Materials Required but not Supplied

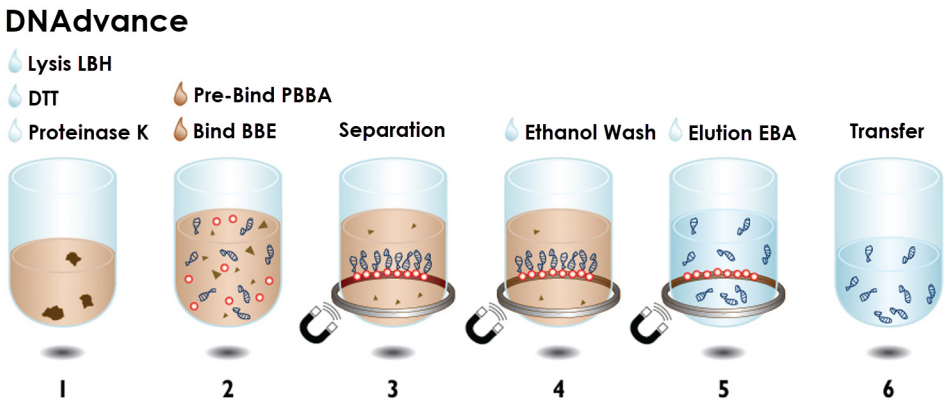
Consumables and Hardware

Item	Type
Magnetic Separator	**SPRIPlate 96R Ring Super Magnet Plate** (Beckman Coulter product # A32782, *www.beckman.com*)
Reaction Plate	**96-well 1.2 mL Magnet Compatible Deepwell Block** (Thermo Scientific product # AB-1127, *www.thermoscientificbio.com*)
Seals	**96 Cap Sealing Mat** (Thermo Scientific product # AB-0662, *www.fishersci.com*)
Incubator Shaker	Any shaking incubator that can be set to 55°C and 100 rpm.

Reagents

Item	Supplier & Catalog Number
100% Ethanol 200 Proof	–
1M DTT	Sigma Aldrich product # 43816, ***or equivalent***

Process Overview



1. Lyse up to 20 mg of specimen in **Lysis** (LBH), **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.

Sample Preparation

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
50 Kit (C42213)	**425 µL**
384 Kit (A48705)	**3.25 mL**
9600 Kit (A48706)	**81 mL**
Storage condition once prepared	**-20°C**

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

a. 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.

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

Procedure (For up to 20 mg of sample)

NOTE Beckman Coulter Inc. strongly recommends using aerosol-barrier (filter) pipette tips when performing the DNAdvance purification.

1 Make the following **Lysis Master Mix**:

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

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

3 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. 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** (LBH).

4 Remove the plate from the water bath or heat incubator.

5 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.

6 Transfer the lysate into a new 1.2 mL plate.

7 Add **100 µL** of **Pre-Bind** (PBBA) buffer and pipette mix **10 times** or until mixed well.

8 Shake **Bind** (BBE) bottle until bead particles are resuspended well in solution.

9 Add **170 µL** of **Bind** (BBE) 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.

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

11 Place the sample plate on an SPRIplate Super Magnet for **4 minutes** to separate the beads from the solution.

12 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.

13 **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.

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

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

Avoid disturbing the ring of magnetic beads.

16 Repeat steps 13 through 15 two more times for a total of three **Ethanol** washes.

17 Remove as much of the final **Ethanol** wash as possible before adding **Elution EBA**.

18 **Take the plate off the magnet.** Add **200 µL** of **Elution** EBA and pipette mix **10 times** or until the magnetic beads are completely resuspended from the bottom of the well.

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

20 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.

21 Store DNA at **-80°C** for further use.

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