Procedure for elution of DNA extracts from

Whatman FTA Elute Cards

Extracted DNA is routinely stored frozen for sample archiving. Whatman™ FTA™ Elute Cards from GE Healthcare can be used to stabilize and protect nucleic acids for long term storage at room temperature. This procedure describes how to apply, store, and elute extracted DNA on FTA Elute Cards.

NOTE: For storage and elution of DNA from biological samples, such as blood and buccal cells, refer to the Instructions for Use for Whatman FTA Elute Cards, Bulletin number 29169169.

Refer to 'Related Documents' section for details of other FTA Elute literature.

Precautions

Handling: Always wear gloves to avoid contamination of FTA Elute Cards. Follow universal precautions when handling biological specimens.

Storage: Samples can be stored in a multi-barrier pouch with desiccant for long term storage.

Materials required

- Indicating FTA Elute Micro Card (WB120411 or WB120412)
- TE^{-4} buffer (10 mM Tris-HCl, 0.1 mM EDTA)
- DNA IQ™ Spin Baskets (V1225 Promega)
- Uni-Core™ Punch 3.0 mm (WB100078 or WB100039)
- Uni-Core™ Punch 6.0 mm (WB100082 or WB100040)
- Cutting Mat (WB100020 or WB100088)
- Heated mixer/shaker
- 1.5 mL microcentrifuge tubes
- 2.0 mL microcentrifuge tubes

Procedure

Applying DNA extract to FTA Elute Cards

1. Label the Indicating FTA Elute Card with the appropriate sample identification.
2. Pipette up to 75 µL of DNA extract onto the card within the printed circle area.
   NOTE: Apply immediately after extraction or vortex prior to spotting.
3. Allow the sample to air dry for at least three hours at room temperature until dry.
4. Once completely dried, store the samples in a cool and dry environment, away from direct sunlight until ready to use. Preferably store in a multi-barrier pouch (WB100036) containing desiccant (10548234).

Elution of DNA from FTA Elute Cards

Refer to Application Note, 29250654 for study data demonstrating that complete STR profiles can be obtained from DNA extracts stored on, and eluted from, Whatman FTA Elute Cards with starting concentrations as low as 50 pg/µL using the following two elution methods.

Method 1. Elution of entire DNA extract (Recommended protocol)

NOTE: Method 1 is recommended for use when elution of the entire DNA extract within the printed circle on the FTA Elute Card is required, regardless of the volume of DNA extract applied (up to 75 µL). This method is often used when concentration of the DNA extract is low (e.g., < 500 pg/µL).

1. Place the FTA Elute Card on a cutting mat.
2. Remove seven, 6 mm punches from the FTA Elute Card and place the punches into a single 2.0 mL microcentrifuge tube.
   NOTE: Seven, 6 mm punches are sufficient to remove the entire area covered by 75 µL of DNA extract.
   NOTE: For this method, remove seven, 6 mm punches for processing ALL volumes of DNA extract, even if <75 µL was applied to the FTA Elute Card.
3. Pipette 1 mL of TE^{-4} buffer into the microcentrifuge tube containing the 6 mm punches.
4. Close the tube and vortex the microcentrifuge tube for five seconds. Ensure the punches move up into the centre of the microcentrifuge tube when they are vortexed.
   NOTE: If the punches remain at the bottom of the microcentrifuge tube during vortexing, they may not be washed adequately.
5. Pipette off excess TE^{-4} buffer and discard.
   NOTE: Remove ALL excess buffer between wash steps.
6. Repeat steps 3–5 (for a total of three washes with TE^{-4} buffer).
7. Pipette 400 µL of TE^{-4} buffer into the microcentrifuge tube containing the sample punches.
   NOTE: This addition of TE^{-4} will serve as the elution volume.
8. Place the microcentrifuge tube on a heated mixer/shaker at 95° C for 30 min at 1,000 rpm.
9. After incubation, briefly centrifuge the microcentrifuge tube to remove any excess liquid from the cap.
10. Place a clean spin basket into a new microcentrifuge tube. Transfer the punches and eluate to the spin basket and spin at maximum speed for two minutes.
11. Remove the spin basket, discard the punches, and proceed with quantification and/or amplification.
    NOTE: If the sample is too dilute to meet the DNA input needed for PCR amplification, the sample can be concentrated.
12. Store extracts according to your laboratory protocols.

Calculation of DNA recovery using Method 1

When using Method 1, calculate % DNA recovery as follows:

$$\% \text{ DNA recovery} = 100 \times \frac{(A \times B)}{(C \times D)}$$

Where:

A = Concentration of DNA eluted from FTA Elute Card (pg/µL)

B = Volume of DNA extract eluted from FTA Elute Card (µL)

C = Concentration of DNA applied to FTA Elute Card (pg/µL)

D = Volume of DNA extract applied to FTA Elute Card (µL)

Method 2. Elution of partial DNA extract

NOTE: Method 2 is recommended for use when the DNA extract stored on the FTA Elute card is not all required for processing, it enables a portion of the sample area to be processed using a variable number of 3 mm punches.

This method is often used when some of the DNA extract is to be retained for future use, or when higher concentrations of DNA extract are stored on FTA Elute cards (e.g., >500 pg/µL).

1. Place the FTA Elute Card on a cutting mat.
2. Remove four, 3 mm punches from the FTA Elute Card and place the punches into a single 1.5 mL microcentrifuge tube.
   NOTE: One to four punches can be used based on known sample concentration and DNA input required for amplification.
   NOTE: Remove punches from the centre of the sample area if possible.
3. Pipette 500 µL of TE^{-4} buffer into the microcentrifuge tube containing the 3 mm punches.
4. Close the tube and vortex the microcentrifuge tube for five seconds.
5. Pipette off excess TE^{-4} buffer and discard.
   NOTE: Remove ALL excess buffer between wash steps.
6. Repeat steps 3–5 (for a total of three washes with TE^{-4} buffer).
7. Pipette an appropriate amount of TE^{-4} buffer into the microcentrifuge tube containing the sample punches based on the number of punches and suggested volumes in Table 1.
   NOTE: This addition of TE^{-4} will serve as the elution volume.

8. Place the microcentrifuge tube on a heated mixer/shaker at 95° C for 30 min at 1,000 rpm.
9. After incubation, briefly centrifuge the microcentrifuge tube to remove any excess liquid from the cap.
10. Place a clean spin basket into a new microcentrifuge tube. Transfer the punches and eluate to the spin basket and spin at maximum speed for two minutes.
11. Remove the spin basket, discard the punches, and proceed with quantification and/or amplification.
    NOTE: If the sample is too dilute to meet the DNA input needed for PCR amplification, the sample can be concentrated.
12. Store extracts according to your laboratory protocols.

Table 1.

Elution volume	Minimum number of punches
>50 µL	1
75 µL	2
100 µL	3
125 µL to 150 µL	4
Increase the elution volume by 25 µL for every additional 3 mm punch	

Sample concentration may be required for any combination of punches and elution volumes depending on the starting concentration of the sample.

Calculation of DNA recovery using Method 2

When using Method 2, calculate % DNA recovery as follows:

1. Measure the diameter of the sample area (stain) covered by the DNA extract and halve this value to calculate the radius (mm).
2. Calculate the area of the stain as follows:

   Area of stain (mm^2) = 3.14 × radius of stain (mm) × radius of stain (mm)

   e.g., for a sample area with a 10 mm diameter:

   Area of 10 mm stain (mm^2) = 3.14 × 5 mm × 5 mm = 78.5 mm^2
3. Calculate quantity of DNA per mm^2 of stain as follows:

   Amount of DNA spotted onto FTA Elute card (pg)/area of stain (mm^2)

   e.g., for a 25 µL sample @ 1,000 pg/µL applied:

   25,000 pg DNA applied per 78.5 mm^2 = 25,000 pg /78.5 mm^2

   Quantity of DNA per mm^2 of stain = 318.47 pg DNA/mm^2
4. Calculate the area of a 3 mm punch

   Area of 3 mm punch (mm^2) = 3.14 x radius of punch (mm) x radius of punch (mm)

   Area of 3 mm punch (mm^2) = 3.14 x 1.5 mm x 1.5 mm

   Area of one, 3 mm punch = 7.065 mm^2

   (Area of two, 3 mm punches = 2 x 7.065 mm^2 = 14.13 mm^2)
5. Calculate quantity of DNA per 3 mm punch:

   If DNA was applied to the FTA Elute card at 318.47 pg/mm^2 and the area of each 3 mm punch is 7.065 mm^2, then,

   Each 3 mm punch should contain:

   318.47 pg DNA/mm^2 × 7.065 mm^2

   Quantity of DNA per 3 mm punch = 2,250 pg

   (Multiply 2,250 pg by number of 3 mm punches processed)
6. Calculate % recovery:

   $$\% \text{ DNA recovery} = 100 \times \frac{(A)}{(B)}$$

   Where:

   A = quantity of DNA eluted from FTA Elute Card (total pg)

   B = expected quantity of DNA per 3 mm punch, calculated in step 5 (pg)

Ordering information

Product	Quantity	Product code
Indicating FTA Elute Micro Card	100	WB120411
Indicating FTA Elute Micro Card	25	WB120412
Uni-Core Punch 3.0 mm	4	WB100039
Uni-Core Punch 3.0 mm	25	WB100078
Uni-Core Punch 6.0 mm	4	WB100040
Uni-Core Punch 6.0 mm	25	WB100082
Cutting Mat 2.5 × 3 in	1	WB100088
Cutting Mat 6 × 8 in	1	WB100020
Multi-Barrier Pouch, small	100	WB100036
Desiccant Pack	100	10548234

Related documents

Title of FTA Elute literature	Bulletin number	Description	Sample type
Whatman FTA Elute Cards	29169169	General Instructions for Use	Biological samples, including blood and buccal cells
Recovery of extracted DNA stored on Whatman FTA Elute Cards	29250654	Study showing effective recovery of extracted DNA stored on Whatman FTA Elute cards at room temperature	Purified DNA, including forensic DNA extracts from crime scene samples
Analysis of DNA on Whatman FTA Elute after 12 years room temperature storage	29250830	Stability study	Blood and purified DNA
Whatman FTA Elute Data File	28984402	Study demonstrating use of DNA eluted from blood-spotted FTA Elute in the following downstream applications: Real Time PCR, Whole Genome Amplification, Sequencing, Multiplex Gene Deletion Assay, ARMS Scorpions Genotyping and Scorpions ASO Genotyping.	Blood

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