



Whatman FTA Protocol BD10

Purification Protocol for Downstream RFLP Analysis of Sample DNA on an FTA[®] Card

FTA[®] Technology

FTA Cards are impregnated with a patented chemical formula that lyses cell membranes and denatures proteins on contact. Nucleic acids are physically entrapped, immobilised and stabilised for storage at room temperature. FTA Cards protect nucleic acids from nucleases, oxidation, UV damage and microbial and fungal attack. Infectious pathogens in samples applied to FTA Cards are rendered inactive on contact. Samples collected on FTA Cards and enclosed in a multi-barrier pouch can be shipped through the post making them an extremely useful tool for field collection of blood, plants or other specimens.

Indicating FTA Cards turn from pink to white on sample application and are recommended for clear or colourless samples. CloneSaver[™] Cards are optimised for the room temperature collection and storage of plasmid DNA.

Handling Instructions

- Always wear gloves when handling FTA or CloneSaver Cards to avoid contamination of the Cards.
- Store unused FTA/CloneSaver Cards in a cool, dry place (avoid light and excessive humidity).
- Follow universal precautions when working with biological samples.
- FTA/CloneSaver Cards are non-toxic to humans.

Materials Required

- Whatman FTA Card – Indicating FTA Cards are recommended for use with clear samples. If applied to non-Indicating Cards, circle the application spot with a ballpoint pen or pencil.
- Whatman FTA purification reagent (cat no. WB120204).
- 6.0mm or 7.0mm diameter paper punch.
- TE⁻¹ buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0).
- Proteinase K (10mg/mL) or DNAzol[™] Reagent (Invitrogen).
- Stock of Restriction Endonuclease of choice (10 U/ μ L).
- 10X restriction digest buffer.
- Sterile de-ionised water.
- 2.0mL microcentrifuge tube with spin basket insert.
- Microcentrifuge capable of speeds up to 12,000 x g.
- 37°C and 60°C incubator.
- Whatman FTA Protocol BD09 “Removing a Sample Disc from an FTA or CloneSaver Card for Analysis”.

The FTA Principle – Get it Right First Time, Every Time

FTA works by lysis of cells releasing the nucleic acid within the matrix of the Card, where the nucleic acid will be entrapped among the cellulose fibres. Therefore the key step to ensure success is getting DNA-containing cells into the FTA in the presence of moisture to activate the cell-lytic and DNA-protective chemicals.

The processing of FTA works by washing away all cell debris and inhibitors of downstream analysis, leaving the DNA immobilised in the cellulose fibres. It is therefore essential that a good wash protocol is followed. Note: a good wash can be visualised in the processing of coloured samples such as blood and plants, where all of the red or green colour would have been removed from the punch. Insufficient washing can mean failure of your downstream analysis.

Controls

It is recommended that internal standard controls are used during each PCR analysis, these should include the following:

- Negative control.
- Negative control with washed, no-sample punch, to ensure that the punch does not cause a positive result.
- Positive control of a known DNA standard solution.
- Positive control standard added to a normally-washed, no-sample punch, to ensure that the punch does not inhibit the reaction.

Protocol

1. Take a sample disc from the dried spot following the instructions provided in the protocol entitled "Removing a Sample Disc from an FTA or CloneSaver Card for Analysis", protocol number BD09. For RFLP analysis a 6.0mm or 7.0mm disc is recommended. Place it in the bottom of the basket insert. Make sure the disc is firmly in contact with the basket.
2. Place the basket into the 2.0mL microcentrifuge tube.
3. Add 500µL FTA Purification Reagent to the basket. Make sure the disc is on the bottom of the basket.
4. Incubate for 1 minute at room temperature.
5. Centrifuge at 6000 x g for 30 seconds.
6. Remove the basket and decant used Reagent.
7. Return the basket to the microcentrifuge tube and repeat Steps 3-6 twice for a total of 3 washes with the FTA Purification Reagent.
8. Add 495µL FTA Purification Reagent and 5µL Proteinase K (10mg/mL) to each tube.
Note: 500µL of DNAzol Buffer can be substituted for the above buffer combination.
9. Incubate for 1 hour at 60°C.
Note: if using DNAzol Buffer, instead of Proteinase K, incubate for 5 minutes at room temperature.
10. Centrifuge at 6000 x g for 30 seconds.
11. Remove the basket and decant used reagent/buffer.
12. Return the basket to the microcentrifuge tube.
13. Add 500µL of TE⁻¹ Buffer to the tube.
14. Centrifuge at 6000 x g for 30 seconds.
15. Remove the basket and decant used buffer.
16. Return the basket to the microcentrifuge tube.
17. Repeat steps 13-16 twice for a total of 3 washes with TE⁻¹ Buffer.
18. Prepare a 1X restriction digest master mix for a final volume of 50µL:
For every 50µL reaction combine:
 - 5µL of 10X restriction digestion buffer.
 - 50 units of restriction enzyme (typically 5µL of a 10U/µL stock).
 - Bring volume up to 50µL by adding sterile, ddH₂O.
19. Place basket in a clean 2mL tube.
20. Add 50µL of master mix to each basket.
21. Incubate for 2 hours at 37°C.
22. Centrifuge at 12,000 x g for 2 minutes.
Note: The restricted DNA is recovered in the microcentrifuge tube.
23. Collected DNA can now be analysed by desired RFLP methods.

Technical Help

If you experience any problems with this protocol or wish to obtain additional information please contact Whatman Technical Service Team on the following regional numbers. Alternatively, please visit www.whatman.com for additional product information and further contact details.

North America 1-800-WHATMAN

Europe +44(0)1622 676670 – ask for technical service

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For Additional Protocol Information Please Visit
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