Whatman FTA Protocol BD08

Preparing an FTA® Disc for DNA Analysis

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).
- TE^{-1} buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0).
- 1.2mm or 2.0mm diameter Harris micro punch or other paper punch.
- Multi-barrier pouch (cat no. WB100010 – large, WB100011 – small).
- Desiccant pack for glycerol stock or high humidity storage areas (cat no. WB100003).
- 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.

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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.
2. Place disc in PCR amplification tube.
3. Add 200µL of FTA Purification Reagent to PCR tube.
4. Incubate for 5 minutes at room temperature (the tube should be given moderate manual mixing to disrupt the debris and aid in washing).
5. Remove and discard all used FTA Purification Reagent using a pipette.
6. For blood or plant presses, repeat steps 3-5 twice, for a total of 3 washes with FTA Purification Reagent. For bacterial samples, repeat steps 3-5 once, for a total of 2 washes with FTA Purification Reagent.
7. Add 200µL of TE^{-1} Buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0).
8. Incubate for 5 minutes at room temperature.
9. Remove and discard all used TE^{-1} Buffer with a pipette.
10. Repeat steps 7-9 once for a total of 2 washes with TE^{-1} Buffer.
11. Ensure that all the liquid has been removed before performing analysis. The disk may be allowed to dry.

It is recommended that analysis be conducted within 3 hours of the disc washing. If this is not possible, the punch can be stored at 4°C or –20°C in a dark environment for up to 1 week.

Preparation for Analysis

PCR

- The washed and air-dried (optional) disc is now ready for analysis by PCR using standard protocols.
- The disc is included in the PCR reaction.
- There is no need to change reaction volume or PCR conditions due to the presence of the disc.
- For the PCR it can be safely assumed that the punch + DNA constitutes zero added volume.
- For PCR analysis of Genomic DNA on a 1.2mm or 2.0mm disc of FTA, on FTA a reaction volume between 25-50µL is recommended.
- For some species of plant a 2.0mm punch may contain too much DNA causing the PCR reaction to be inhibited. In this situation a 1.2mm punch can be used in as much as 50µL of PCR reaction mix.

STR (Short Tandem Repeats)

- The washed and air-dried (optional) disc is now ready for STR analysis. A 1.2mm disc contains about 5-20ng DNA. Accordingly, an appropriate cycle number for this high quantity of DNA is 24 cycles, the amplification is performed directly in the MicroAmp tube.
- STR profiles have been successfully generated with 1.2mm FTA discs using kits from Applied BioSystems and Promega.

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
Japan +8(0)3 3832 6707 – ask for technical service
Asia Pacific +65 6534 0138 – ask for technical service
China +86 21 6443 7176 – ask for technical service
India +91 22 529 7035 – ask for technical service

For Additional Protocol Information Please Visit www.whatman.com

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