



Whatman FTA Protocol BD05

Applying and Preparing Plant Samples on FTA[®] Cards 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. Plant samples can be applied to FTA cards in two ways: 1) Applying a leaf press and 2) Applying a plant tissue homogenate (for details see below).

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.
- Blunt instrument such as a hammer or leaf press for pressing the leaf tissue or mortar and pestle for grinding leaf tissue to create a homogenate.
- 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.

Protocol

Sample Collection by Direct Leaf Press

1. Label the FTA Plant Card with the appropriate sample identification.
2. Place the leaf over the marked circle (underside of the leaf facing down) on top of the FTA Matrix Card.
3. Overlay the leaf, either with Parafilm or replace the cover sheet.
4. Using a heavy blunt object (such as a small porcelain pestle, tack hammer or screwdriver handle) pound each sample area with moderate force for 15 seconds. This will burst the cell walls of the plant tissue.
5. Alternatively, the sample may be pressed onto the FTA using pliers with smooth surface. It is important that the samples be pressed by a smooth surface so that the filter matrix is not damaged.
6. Verify that sufficient plant material has been transferred to the paper by checking the back of the FTA Card: plant tissue should be visible on the other side of the matrix.
7. **Note:** Use great care not to damage the matrix.
8. Ensure that no large pieces of plant tissue remain adhered to the FTA Card as this may interfere with later processing.
9. When samples have been transferred to the paper allow the FTA Card to air dry for a minimum of 1 hour at room temperature.
10. If sample is to be archived, place in a multi-barrier pouch with desiccant or store in a humidity controlled, cool, dry environment.

Helpful Hints: A quality leaf press is the most important factor for obtaining good quality plant DNA sample on the FTA Card. A leaf pressing that is made with too little force will not transfer enough DNA to the Card. For the best results it is important to apply quick, strong pressure to the parafilm/leaf/card sandwich. This will provide enough momentum to break the cell walls. Applying too much or too little pressure or pressure that is uneven is not likely to work well. Also, hitting the Card too strongly will damage the matrix making it too fragile for processing. When applying force, do not use a rubbing motion.

Sample Collection of Plant Homogenate

1. Label the FTA Card with the appropriate sample identification.
2. Use a minimum of 10mg of young plant tissue. Add 1 part plant tissue to 5 parts PBS and using a mortar and pestle grind leaf material to a smooth homogenate (if preferred use a micropestle and microfuge tube). The ratio of 1 part plant material and 5 parts PBS is critical for good results. For soybean and some species of cereal it may be necessary to add dithiothreitol (DTT) to improve the amount of DNA that binds to the FTA.

3. Apply the homogenate to the FTA Classic Card matrix inside the marked circle using a wide-mouth pipette or a pipette tip that has been cut to give it a 1.5 to 2.0mm opening (the sample will likely be too viscous to use pipette tips with narrow openings). Allow the sample to air dry on the FTA Card for a minimum of 2 hours at room temperature.
4. If all of the plant tissue can not be homogenised completely, the semi-homogenised tissue can be pressed against the Card and then discarded.
5. If sample is to be archived, place in a multi-barrier pouch with desiccant or store in a humidity controlled, cool, dry environment.

Preparing the FTA for DNA Analysis

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 plant samples a 2.0mm disc is recommended.)
2. Place disc in PCR amplification tube (or 1.5mL microcentrifuge tube).
3. Add 200µL of FTA Purification Reagent to PCR tube.
4. Incubate for 5 minutes at room temperature with moderate manual mixing.
5. Remove and discard the used FTA Purification Reagent with a pipette.
6. Repeat steps 3-5 once, for a total of 2 washes with FTA Purification Reagent.
7. At this stage the FTA disc should be white (ie no chlorophyll). If the disc is still very green repeat steps 3-5 for an additional FTA Reagent wash.
8. Add 200µL of TE⁻¹ Buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0).
9. Incubate for 5 minutes at room temperature.
10. Remove and discard all used TE⁻¹ Buffer with a pipette.
11. Repeat steps 8-10 once for a total of 2 washes with TE⁻¹ Buffer.
12. 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.

Additional Method: Modified Protocol for Enhanced Sample Washing

With some plant species removal of chlorophyll from the punch becomes difficult. This may interfere with downstream analysis. The following protocol details an enhanced washing method developed to remove the chlorophyll from such samples:

1. After washing with the FTA Purification Reagent, wash the punches with 200µL isopropanol.
2. Incubate for 2 minutes, pipette up and down a couple of times and discard. Repeat for a total of 2 washes with isopropanol.
3. Dry the punches at room temperature to ensure that the isopropanol has been completely removed.

DNA Analysis of Samples on FTA

PCR

- The washed and air-dried 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.
 - Recommended reaction volume of the PCR analysis of Plant DNA is between 25-50µL.
 - 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.
-

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