MatMaCorp

StickE™ Column Plant DNA Isolation Kit

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Patent Pending

Introduction

MatMaCorp's StickE™ Column Plant DNA Isolation Kit offers a quick and simple DNA isolation procedure that requires a small amount of sample material to isolate high quality DNA. DNA from the lysed sample is bound to the StickE™ Column and the binding is reversed in the elution step, yielding DNA for use in any application such as PCR, genotyping, or MatMaCorp's C-SAND™ assays.

This kit allows the user to process samples in about 10 minutes. DNA can be isolated from less than 60mg of sample from a variety of species.

Samples from the following species have been successfully tested:

- Corn
- Wheat
- Milo
- Potato
- Hemp
- Soybean
- Arabidopsis
- Tomato
- Strawberry

Kit storage

The StickE™ Column Plant DNA Isolation Kit can be stored at room temperature. Exposing components of this kit to high temperatures, (above 90°C) and freezing should be avoided. Use of this kit is not recommended after the expiration date.

Disclaimer

This product has been developed and designed for research purposes only. It is not intended for diagnostic use. Material Safety Data Sheets (MSDS) for all StickE™ Column Kits can be found at *www.matmacorp.com.*

NOTE: PLEASE READ THIS ENTIRE MANUAL INCLUDING THE PREPARATION STEPS AND THE DETAILED PROCEDURE BEFORE BEGINNING THE PROTOCOL.

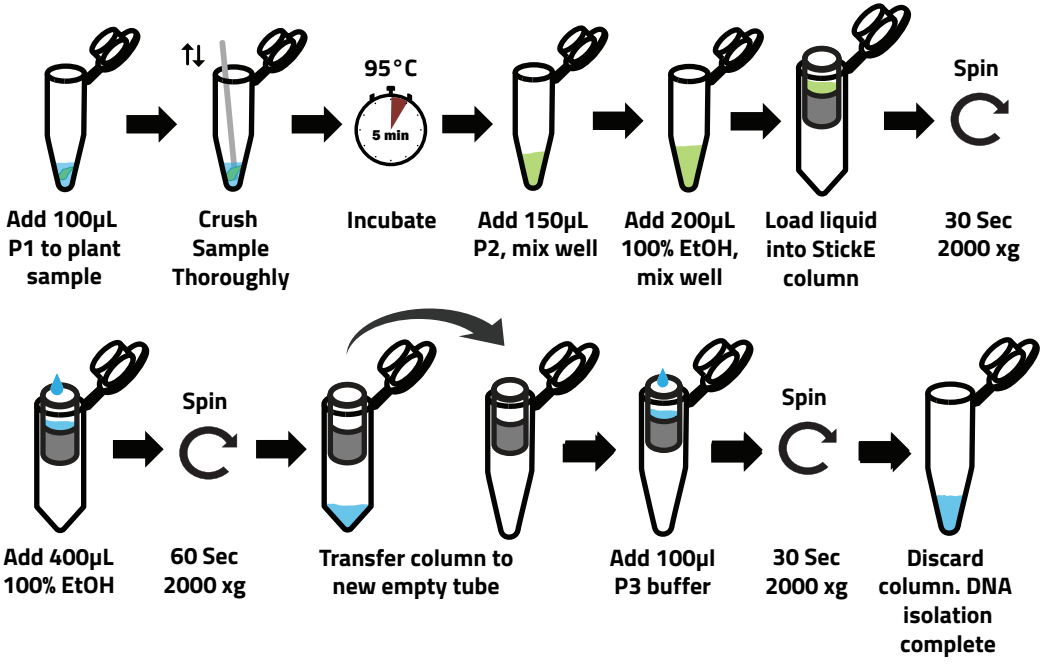
Kit Contents

50 of each:
2 mL tubes with StickE™ Column inserted
Crushing rods

1 of each:
Bottle of Lysis Buffer (P1)
Bottle of Binding Buffer (P2)
Bottle of Elution Buffer (P3)

Overview

This overview is only intended as a quick reference guide. Please read this entire manual before beginning the protocol.



StickE™ Column Plant DNA Isolation Protocol

This protocol has been optimized to isolate DNA from fresh or frozen plant samples. For DNA isolation from other sample types, please use the appropriate StickE™ Column Kit. Please be aware that the procedure below is for a single sample.

Materials needed by user:

- Solas 8® (If not available, a heat block capable of 95°C and compatible with 1.5mL centrifuge tubes will suffice).
- Mini centrifuge or any centrifuge capable of 2000 xg and compatible with 1.5mL centrifuge tubes.
- 100% Ethanol
- 1.5mL centrifuge tubes

Preparation:

If using the Solas 8® device:

a. Set up a user profile and enter sample IDs for the samples desired.
b. Start the DNA isolation procedure on the Solas 8® for the selected sample.
c. Once the initial instruction of the protocol on the Solas 8® is marked as completed the heat block will begin heating.
d. Two 1.5mL tubes and one 2mL tube with inserted StickE™ column are needed for each sample, label these with the sample ID.

If not using Solas 8®:

a. Set a heat block to 95°C.
b. Two 1.5mL tubes and one 2mL tube with inserted StickE™ column are needed for each sample, label these with the sample ID.

Procedure:

(these instructions will also appear on the screen of the Solas 8®)

NOTE: Gloves are recommended to prevent contamination.

1. Into a 1.5mL tube, add a plant sample that is 60 mg or equal to eight 2 mm punches.

2. Add 100µL P1 buffer to sample.

Procedure (Continued):

3. Crush the sample thoroughly and repeatedly using the supplied crushing rod. Crush the sample using a rapid up and down motion.

4. If using the Solas 8®, when the temperature reaches 95°C, the start button will be activated. Place tube containing sample into the Solas 8® and press start to initiate a 5-minute incubation.

   If using a heat block, place tube into block once the 95°C temperature has been reached. Incubate for 5 minutes.

5. Remove the tube from heat (Repeating the crushing and heat steps may increase yield), gently mix by flicking tube or by a brief vortex and place in tube rack. Discard crushing rod.

6. Add 150µL P2 buffer to sample and gently mix by flicking the tube lightly, or by a brief vortex.

7. Add 200µL 100% Ethanol to sample mixture and gently mix by flicking the tube lightly, or by a brief vortex.

8. Carefully apply the lysate into a StickE™ column and close the lid of the tube. Do not load any visibly solid material into the column as this may affect elution.

9. Place the tube with the StickE™ column into a mini centrifuge.

10. Spin briefly (about 30 seconds) at 2000 xg. If all liquid has not passed through column, continue spinning until all the liquid has passed through the column.

11. Open tube and add 400µL 100% Ethanol.

12. Close tube and return it to the centrifuge.

13. Spin briefly (about 30 seconds) at 2000 xg. If all liquid has not passed through column, continue spinning until all the liquid has passed through the column.

14. Discard flow through and replace the collection tube.

14. Spin again for 60 seconds at 2000 xg to dry StickE column.

15. Remove the StickE™ column from its tube and place into a new 1.5mL centrifuge tube (provided by the user). The tube containing the flow through may be discarded.

Procedure (Continued):

16. Add 100µL P3 buffer into the top of StickE™ column.
17. Close lid of tube firmly and place into a mini centrifuge.
18. Spin 30 seconds at 2000 xg.
19. The StickE™ column may now be discarded.
20. The 1.5mL centrifuge tube contains DNA from the loaded sample. Your DNA isolation is complete.

Troubleshooting

Problem	Cause	Solution
Low Yield	Amount of sample too low or high	Optimal amount of tissue sample is 60mg, adjust sample size.
	Incorrect temperature	The Solas 8® preheating takes place once the first step of the protocol is selected on the screen. Wait until the device has reached desired temperature before loading samples. Check if heat block is operating at proper temperature (95°C).
	Excess elution buffer	Over-diluted DNA from excess elution buffer can be concentrated with evaporation.
Assay Interference	Buffer contamination	StickE™ columns should be spun on a mini centrifuge to clear buffers before elution, increase spin time if problem persists.
	Excess DNA	Dilute the elution 1:10
Low Purity	Amount of sample is too high	If the starting material is more than 60 mg, scale up the volume of buffers accordingly. Dilute the elution 1:10
	Ethanol contamination	StickE™ columns should be spun on a mini centrifuge for at least 60 sec to clear ethanol, longer if problem persists.

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