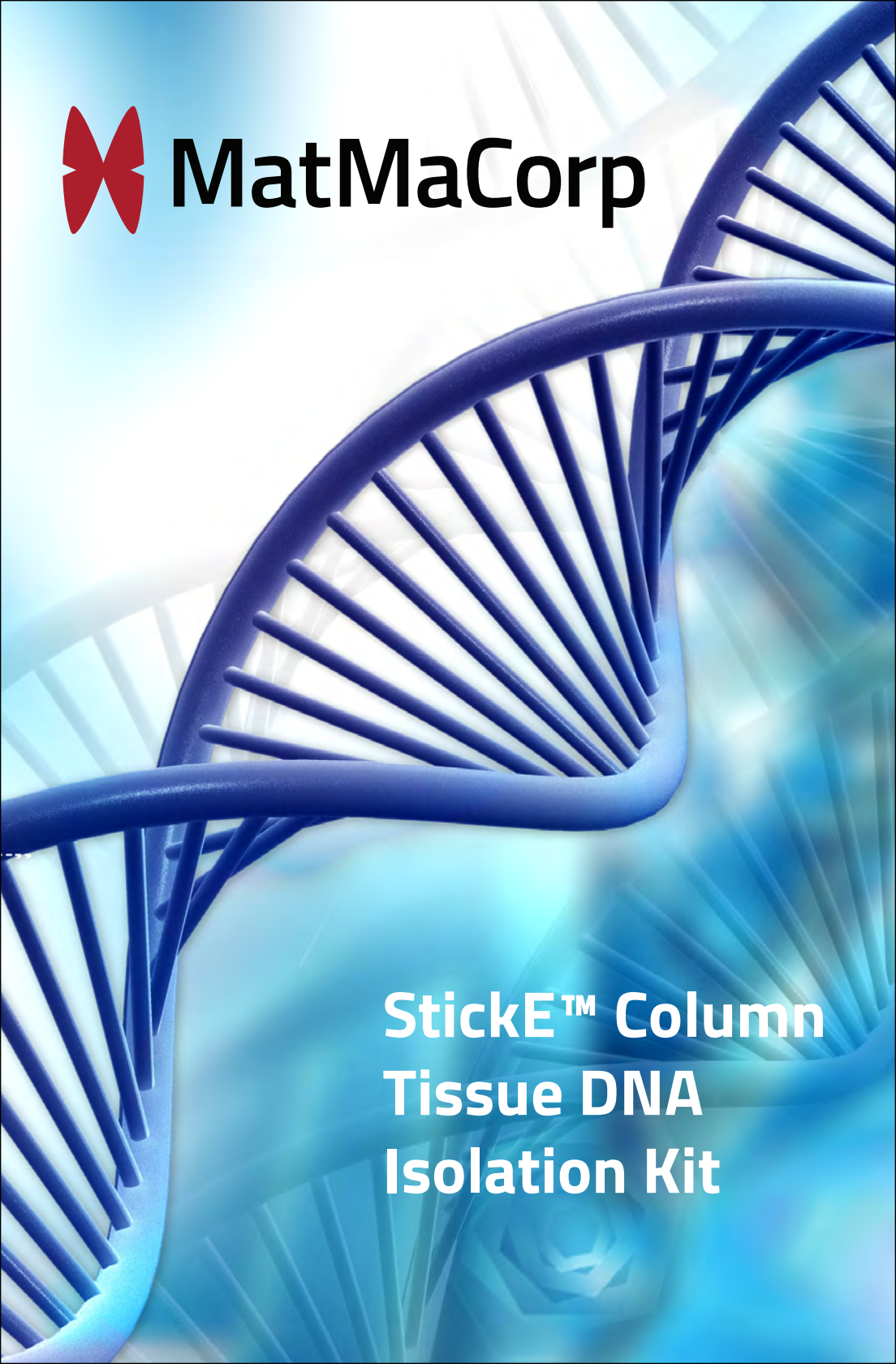
MatMaCorp



StickE™ Column Tissue DNA Isolation Kit

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Manual: 1st Edition - January 2021

Patent Pending

Introduction

MatMaCorp's StickE™ Column Tissue 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 20mg of tissue from a variety of sources including muscle, skin, organs, etc. This kit can also process farm animal ear tag punches from the AllFlex TSU collection system.

Tissue from the following species have been successfully tested:

- Chicken
- Cow
- Pig
- Fruit Fly
- Turkey
- Zebrafish
- Rabbit
- Mouse
- Dog

Kit storage

The StickE™ Column Tissue 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.

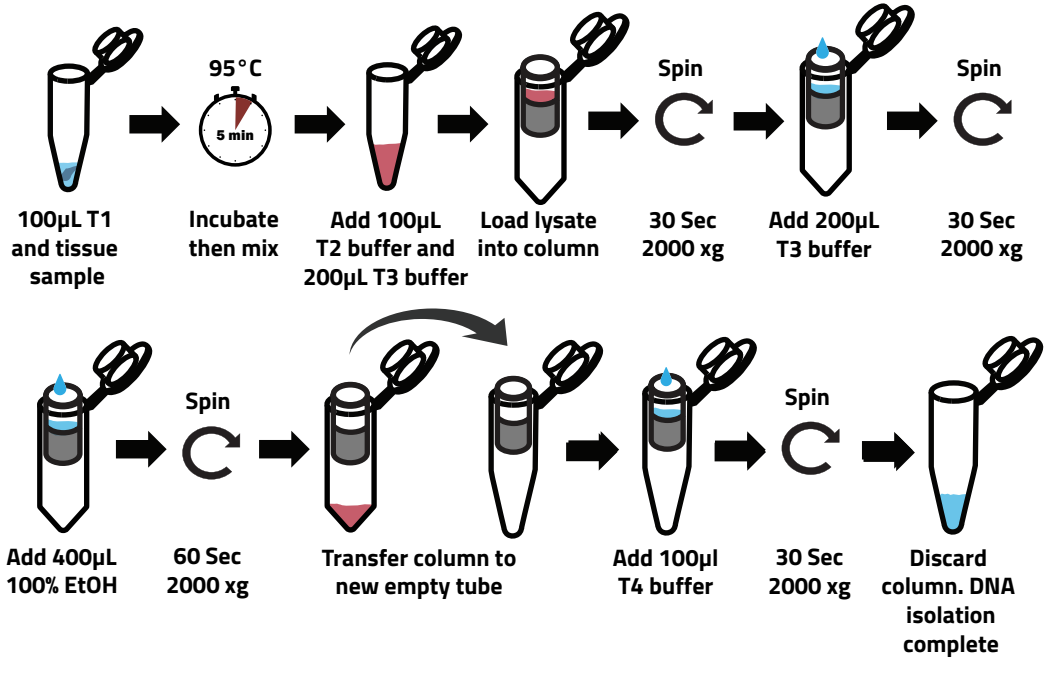
Kit Contents

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

1 of each:
Bottle of Lysis Buffer (T1)
Bottle of Binding Buffer (T2)
Bottle of Wash Buffer (T3) - See preparation step 1
Bottle of Elution Buffer (T4)

Overview

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



StickE™ Column Tissue DNA Isolation Protocol

This protocol has been optimized to isolate DNA from fresh or frozen tissue 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:

1. Add 16.5mL of 100% Ethanol to the T3 buffer bottle. **(This step only needs to be completed once)**

NOTE: To avoid repeating this step, it is advised to check the box on the bottle label to indicate that this step was completed (see graphic).

2. 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.

3. If not using Solas 8®, set a heat block to 95°C.

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 tissue that is approximately 20 mg, or about the size of this dot: ● (sample density may affect this size) Small insects may require more than one individual. Grinding the insect material with a grinding tool helps increase yield.

2. Add 100µL T1 buffer to tissue sample.

Procedure (Continued):

3. If using the Solas 8®:

    a. When the temperature reaches 95°C, the start button will be activated. Place tube containing sample into the Solas 8® and press start.
    b. Timer will countdown incubation time.

    If using a heat block:

    a. Place tube at 95°C for 5 minutes.

4. Remove the tube from heat, gently mix by flicking tube or briefly vortex. Many tissue samples dissolve completely, but some sample types may not totally dissolve.

5. Add 100µL T2 buffer to sample.

6. Add 200µL T3 buffer to sample, vortex briefly to mix.

7. Apply the entire lysate mixture to the StickE™ column (in collection tube) and close the lid of the tube. Do not load any visibly solid tissue material into the column.

8. Spin for 30 seconds at 2000 xg.

9. Discard flow through, then place the StickE column back into collection tube.

10. Add 200uL T3 Buffer to column.

11. Spin for 30 seconds at 2000 xg.

12. Discard flow through, then place the StickE column back into collection tube.

13. Add 400µL of 100% EtOH to StickE™ column.

14. Spin for 30 seconds at 2000 xg.

15. Discard flow through, then place the StickE column back into collection tube.

Procedure (Continued):

16. Spin for 60 seconds at 2000 xg.

17. 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.

18. Add 100µL T4 buffer into the StickE™ column.

19. Spin 30 seconds at 2000 xg.

20. The StickE™ column may now be removed and discarded.

21. 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 20mg, adjust sample size.
	Incorrect temperature	Check temperature of heat block or Solas 8®. Kit will perform between 70°C - 95°C, the temperature for optimal performance is 95°C.
	Incomplete lysis	Repeat isolation with fresh sample in the correct volume of lysis buffer T1 (100µL). Lyse at 95°C for 5 minutes.
	Overheating	Heating longer than 10 minutes significantly reduces yield, repeat isolation.
Assay Interference	Ethanol contamination	StickE™ columns should be spun on a centrifuge for 60 sec to clear ethanol before elution step.
	Steps completed in incorrect order	Complete steps in the order listed.
Low Purity	Amount of sample is too high	Optimal amount of tissue sample is 20mg, adjust the sample amount. If sample DNA is already isolated, dilute the elution 1:10.
	Excess T2 buffer added	If the buffer in excess is T1, the same volume of T2 buffer can be added with limited effect. An excess of T2 buffer cannot be resolved.
	Incomplete washing	Wash bound DNA with ethanol before elution.

MatMaCorp, Solas 8, and StickE are trademarks of Stem Arts Projects, LLC, USA. AllFlex is a registered trademark of AllFlex, USA.

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