
MatMaCorp
StickE™ Column
Blood DNA
Isolation Kit

StickE™ Column Blood DNA Isolation Kit

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Manual: 2nd Edition - November 2023

Patent Pending

Introduction

MatMaCorp's StickE™ Column Blood DNA Isolation Kit offers an efficient, user-friendly procedure to isolate high-quality DNA from nucleated and non-nucleated blood. DNA is first bound to the StickE™ Column during the initial stages, then the binding process is reversed later in the procedure, eluting DNA.

The DNA is suitable for downstream applications such as PCR, RT-PCR, genotyping, or MatMaCorp's C-SAND assays.

Kit storage

The StickE™ Column Blood 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. Safety Data Sheets (SDS) 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.

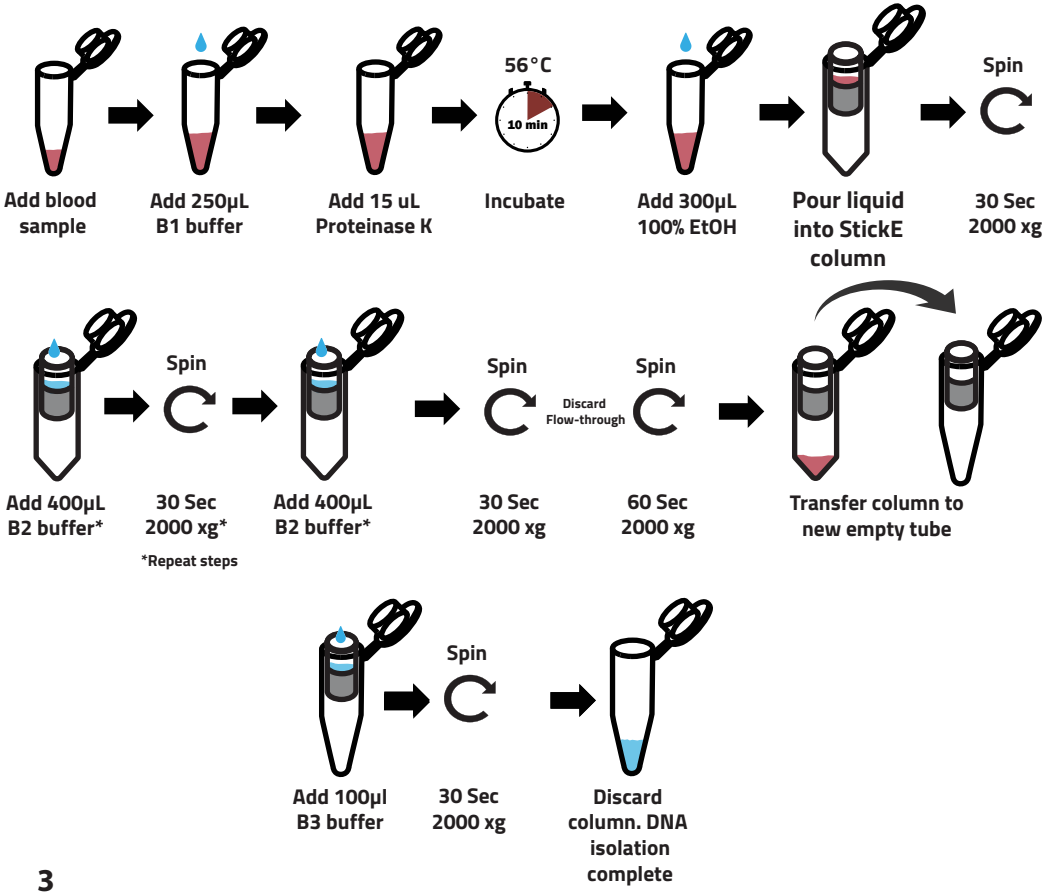
Kit Contents

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

1 of each:
Bottle of Lysis/Binding Buffer (B1)
Bottle of Wash Buffer (B2)
Bottle of Elution Buffer (B3)
Tube of Proteinase K

Overview

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



StickE™ Column Blood DNA Isolation Protocol

This protocol has been optimized to isolate DNA from fresh or frozen anticoagulant-treated blood 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 56°C and compatible with 1.5mL centrifuge tubes will suffice)
- Vortex capable of 3000rpm
- Centrifuge capable of 2000g and compatible with 1.5mL centrifuge tubes
- 100% Ethanol
- 1.5mL centrifuge tubes

Preparation:

1. Add 33mL of 100% Ethanol to the B2 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 56°C.

4. Into a 1.5mL tube, add 100µL of **non-nucleated** blood, or 10-50µL of **nucleated** blood.

Procedure:

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

NOTE: Gloves are recommended to prevent contamination.

1. Add 250µL of B1 buffer to the blood sample.

Procedure (Continued):

2. Add 15 uL Proteinase K to the blood sample.

3. Vortex.

4. If using the Solas 8®:
    a. When the temperature reaches 56°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 56°C for 10 to 15 minutes.

5. Remove the tube from heat.

6. Vortex.

7. Add 300µL of 100% EtOH to sample.

8. Vortex.

9. Apply the entire lysate mixture to the StickE™ column (in collection tube) and close the lid of the tube.

10. Spin for 30 seconds at 2000 xg.

NOTE: If all the sample does not pass through the column, centrifuge at 6000g until the complete sample passes through the column.

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

12. Add 400µL B2 Buffer to the StickE™ column.

13. Spin for 30 seconds at 2000 xg.

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

15. Repeat Steps 12-14.

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

17. Spin for 30 seconds at 2000 xg.

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

19. Spin for 60 seconds at 2000 xg.

20. Remove StickE™ column from collection tube and transfer to a new 1.5mL tube.

21. Add 100µL of B3 buffer to the StickE™ column.

Procedure (Continued):

22. Spin for 30 seconds at 2000 xg.

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

24. 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 non-nucleated blood is 100µL. Optimal amount of nucleated blood is 50µL.
	Incorrect temperature	Check temperature of heat block or Solas 8®. Kit will perform between 50°C - 70°C, the temperature for optimal performance is 56°C.
	Incomplete lysis	Repeat isolation with fresh sample in the correct volume of B1 buffer (250µL).
	Overheating	Heating longer than 20 minutes significantly reduces yield, repeat isolation.
	Incomplete incubation	Allowing all buffers to incubate on the column for 1 minute will increase the yield.
Assay Interference	Ethanol contamination	StickE™ columns should be spun on a centrifuge for 30 sec after the additon of EtOH. The column should then be spun for 60 sec to make sure 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 non-nucleated blood is 100µL. Optimal amount of nucleated blood is 50µL.
	Incomplete washing	Wash bound DNA with two volumes of B2 buffer and one volume of 100% EtOH.
Clogged Column	Insufficient mixing	Each vortex step should last a minimum of 10 seconds.
	Old blood sample	For optimal results, use a fresh blood sample.
	Insufficient centrifuge speed/time	Check centrifuge to ensure the speed is set at 2000g. Follow exact centrifuge times as listed in the protocol. If the problem persists, increase the centrifuge speed to 6000g and repeat centrifuge steps.

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