MatMaCorp

StickE™ Column Semen DNA Isolation Kit

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Manual: 1st Edition - May 2022

Patent Pending

Introduction

MatMaCorp's StickE™ Column Semen 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 18 minutes. DNA can be isolated from less than 25 µl of undiluted semen or 100 µl extended semen.

Kit storage

The StickE™ Column Semen 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.

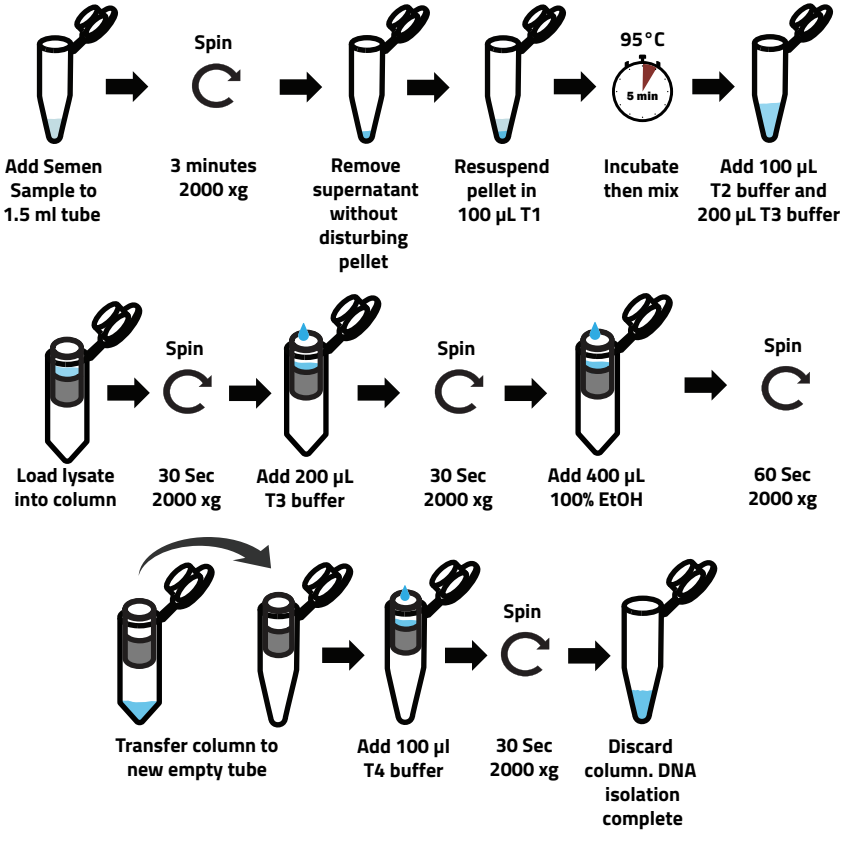
Kit Contents

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 Semen DNA Isolation Protocol

This protocol has been optimized to isolate DNA from fresh or frozen semen 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 1.5 mL centrifuge tubes will suffice).
- Mini centrifuge or any centrifuge capable of 2000 xg and compatible with 1.5 mL centrifuge tubes.
- 100% Ethanol
- 1.5 mL centrifuge tubes

Preparation:

1. Add 16.5 mL 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 1.5 mL tube, add 100 µL of extended semen or 25 µL of undiluted semen.
   a. Add 75 µL of sterile water if using undiluted semen.

2. Spin for 3 minutes at 2000 xg.

Procedure (Continued):

3. Remove supernatant without disturbing the pellet.

4. Add 100 µL T1 buffer to the sample pellet.

5. 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.

6. Remove the tube from heat, gently mix by flicking tube or briefly vortex.

7. Add 100 µL T2 buffer to sample.

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

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.

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

12. Add 200 uL T3 Buffer to column.

13. Spin for 30 seconds at 2000 xg.

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

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

16. Spin for 30 seconds at 2000 xg.

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

18. Spin for 60 seconds at 2000 xg.

Procedure (Continued):

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

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

21. Spin 30 seconds at 2000 xg.

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

23. The 1.5 mL centrifuge tube contains DNA from the loaded sample. Your DNA isolation is complete.

Troubleshooting

Problem	Cause	Solution
Low Yield	Amount of sample too low	Optimal amount of semen sample is about 25 µL to 100 µL, adjust sample size.
	Incorrect Lysis temperature	Check temperature of heat block or Solas 8®. Kit will perform between 85°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 (100 µL). 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 seconds 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 semen sample is about 25 µL to 100 µL, 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.
Clogged Column	Amount of sample is too high	Optimal amount of semen sample is about 25 µL to 100 µL, adjust the sample amount.

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