Isopure™ for Buccal Cell DNA

Catalog Numbers: **P-9050-S, P-9050-M, P-9050-L**

Contents

Protocol Manual *Revision 6.1*

Please refer to http://www.alinebiosciences.com Support section for updated protocols and MSDS when handling or shipping any chemical hazards. The information provided is subject to change without notice.

ALINE BIOSCIENCES
Tel.: 1-888-987-3677 | Web: www.alinebiosciences.com | E-mail: support@alinebiosciences.com

INTRODUCTION

The ALINE Isopure™ Buccal Cell Genomic DNA Kit utilizes ALINE's proprietary paramagnetic bead technology to isolate genomic DNA from buccal cells or pelleted cells from a mouthwash. The protocol may be performed manually or in 96-well format for high throughput DNA extraction. Purification begins by lysing buccal cell with Proteinase K release DNA. DNA is subsequently immobilized on magnetic particles. This differential binding allows DNA to be separated from contaminants using a magnetic plate. Contaminants can then be rinsed away using a simple washing procedure, leaving the genomic DNA ready for elution from the magnetic particles. The yield is from 5-15ng/ul depending the cell numbers. The 96-well plate format procedure is highly amenable to automation due to the utilization of magnetic separation. Vacuum filtration or centrifugation is eliminated.

Genomic DNA prepared from the ALINE Isopure™ Kit can be used in the following applications:

- PCR amplification
- Restriction enzyme digestion
- Human identity testing
- Membrane hybridizations (e.g., Southern and dot/slot blots).
- AFLP, RFLP, RAPD, microsatellite and SNP analyses (for genotyping, fingerprinting, etc.)

Process Overview:

1. Digestion of buccal cells in Lysis Buffer with Proteinase K.
2. Bind genomic DNA to paramagnetic beads.
3. Separate beads from contaminants.
4. Wash the magnetic beads with an ethanol wash buffers to remove contaminants.
5. Elute DNA from magnetic particles.
6. Transfer eluted DNA to a new plate.

Statement of Warnings

The U.S. Centers for Disease Control, the Food and Drug Administration, and the American Hospital Association recommend applying "universal precautions" when handling subject's specimens to protect health care and laboratory workers. Under universal precautions, all subjects are considered potentially infectious for blood-borne pathogens. It is recommended that workers protect themselves from contact with the specimens by wearing Proper Protective Equipment which includes gloves, goggles, and lab coats.

ALINE BIOSCIENCES
Tel.: 1-888-987-3677 | Web: www.alinebiosciences.com | E-mail: support@alinebiosciences.com

 	DANGER	
	Proteinase K	
	H315	Causes skin irritation.
	H319	Causes serious eye irritation.
	H334	May cause allergy or asthma symptoms or breathing difficulties if
	inhaled.	
	H335	May cause respiratory irritation.
	P261	Avoid breathing vapors.
	P280	Wear protective gloves, protective clothing and eye/face protection
	P284	In case of inadequate ventilation, wear respiratory protection.
	P304+P340	IF INHALED: Remove person to fresh air and keep at rest in a position comfortable for breathing.
	P312	Call a POISON CENTER or doctor/physician if you feel unwell.
	P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER or
	doctor/physician.	
	P403+P233	Store in a well-ventilated place. Keep container tightly closed.
		Safety Data Sheet is available at www.beckman.com/techdocs.

.

WARNING
Bind 1: Thiocyanate Sodium 20 – 30%
H303
H313
H412
P273
P312
Safety Data Sheet is available at www.alinebiosciences.com

Starting Material: Buccal cells collected with cytobrush.

Reagents supplied in the kit:

Lysis Buffer (clear)**:** Store at Room Temperature. .
Magnetic beads: Store at 2-8°C
Wash 1 Buffer: Concentrated. Need to add 95-100% ethanol as specified on the bottle before the first use. Store at Room Temperature.
Wash 2 Buffer: Concentrated. Need to add 95-100% ethanol as specified on the bottle before the first use. Store at Room Temperature.
Elution Buffer: Store at Room Temperature.
Proteinase K: Store at -20°C

Reagents to be supplied by users:
96-well plates 1.2mL
100% isopropanol
95-100% ethanol

Note: Make sure to add ethanol to the concentrated Wash Buffers before the first use according to the instructions on the bottle.

Part A. Before start, prepare the following solutions:

1. Prepare Bind Solution by mixing 20uL Magnetic beads with 250uL isopropanol. For 96 well sample, mixing 2.04mL magnetic beads with 25.5mL isopropanol.
2. Prepare Lysis Solution by mixing 35mL Lysis buffer with 1 mL proteinase K.

PART B. PROCEDURE IN 96-WELL FORMAT

ALINE Biosciences strongly recommends using aerosol-barrier (filter) pipette tips when performing the protocol.

1. **Add 360 µl Lysis Solution prepared in Part A into each sample tube.**

ALINE BIOSCIENCES
Tel.: 1-888-987-3677 | Web: www.alinebiosciences.com | E-mail: support@alinebiosciences.com

NOTE: 350 uL Lysis buffer and 10 uL proteinase K may be added into sample tube separately.

Lysis solution may be added directly to pelleted cells from a mouthwash. A 1.2 mL 96-well plate may be used. Care should be taken to avoid cross-contamination.

2. Rotating cytobrush or swab in lysis solution for 10 times to release buccal cells. Press cytobrush/swab against tube wall to release residual solution before discarding cytobrush/swab. Cells will float in solution or sink to the bottom.

NOTE: DNA yield is proportionally related to cell numbers. Multiple cytobrush/swab may be used to increase cell number.

3. Incubate samples at 56ºC for 10 minutes. If a full plate is to be used, extend time to up to 30 minutes

4. Add 200 µL of Bind 1 (conditioning) to each sample.

5. Shake Bind Solution prepared in Part A until bead particles are fully re-suspended. Add 270ul beads to each sample.

6. Place the sample tube/plate on a strong magnet for 5 minutes to separate.

7. Aspirate and discard the supernatant while the tube/plate is situated on the magnet.

NOTE: When aspirating, place the pipette at the center of the well to avoid disturbing magnetic beads.

8. (Optional for improved 260/230 ratio) Take the tube/plate off the magnet. Add 600 µL of Wash 1 Buffer and pipette mix 10 times or until the magnetic beads are re-suspended from the bottom of the well.

9. Place the tube/plate back on the magnet for 3 minutes or until the solution clears.

10. Aspirate and discard the supernatant while the tube/plate is situated on the magnet. Avoid disturbing the ring of magnetic beads.

11. Take the tube/plate off the magnet. Add 600 µL of Wash 2 Buffer and pipette mix 10 times or until the magnetic beads are re-suspended from the bottom of the well.

12. Place the tube/plate back on the magnet for 5 minutes or until the solution clears.

13. Repeat steps 11 through 12 once for a total of two washes. Completely remove any residual liquid after the final wash.

14. Dry the plate at room temperature for 5 minutes.

15. Take the tube/plate off the magnet. Add 50 µL of EB or reagent water and pipette mix 10 times or until the magnetic beads are completely resuspended from the bottom of the well.

NOTE: Preheat water to 60-80ºC helps increase the yield.

15. Place the tube/plate back on the magnet for 3 minutes, or until the solution clears.

16. Transfer 49 µL of supernatant to a clean tube/plate for storage.

NOTE: when using a plate, aspirate slowly and do not disturb the ring of beads while pipetting. Transferring all 50 µL of product is not recommended as it may carry over some magnetic beads. If beads are being aspirated during the transfer, dispense the sample back into the plate and incubate for another 5 minutes and then aspirate slowly.

The DNA can be stored at -20° for further use.

~~~

If you have any technical questions, please feel free to contact support@alinebiosciences.com or 1-888-987-3677.

Disclaimer from ALINE Biosciences:
~~~