PathoID Inc.

Assays from Imagination to Reality

Buccal Cell DNA Extraction Kit

Education and Hobbyist Series

p/n: 020010101

Application: Collection of buccal (cheek swab) samples and subsequent extraction of DNA suitable for molecular analysis.

Kit size: 25 samples.

Storage conditions: Ambient storage. Product stability has not been extensively tested, and no expiration date is specified as of this product revision. Stability under ambient conditions expected to be > 12 months.

Contents:

- 25 Sample Sticks – *treated to remove surface DNA*
- 25 Sample Collection Tubes – *DNase free*
- 3 x 2.0 ml, Lysis Media (blue caps)
- 1 x 100 µl, Lysis Agent (yellow cap) – *volume approximate; >55 µl*
- 1 x 1.0 ml, PCR grade water (white cap) – *tested free of detectable DNase or amplifiable human DNA*

As needed, use a microcentrifuge to collect any reagents at bottom of tube before use.

User Equipment Required:

- Micropipettors and pipette tips (1 ml, 200 µl, and 10 µl sizes suggested)
- Microcentrifuge with rotor for 1.5 ml MFT; capable of 12,000 rpm
- 91% Isopropanol *Available at pharmacies, may be labelled as Rubbing Alcohol. Use of commonly available 70% isopropanol instead of 91% is not recommended and may lead to poor DNA recovery.*
- Foam float for tubes
- Dish/ pot/ beaker with boiling or near boiling water.

Protocol:

1. Wear appropriate PPE and take standard laboratory precautions when using this kit. In particular, wear disposable lab gloves when handling Sample sticks and Collection tubes.
2. Avoid contamination of the sample or collection tubes with exogenous DNA as much as practical for your setting.
3. For each sample to be processed, get and label one Sample Collection Tube.
4. Add 200 µl Lysis Media (blue cap) to each labelled Sample Collection Tube.
5. Remove a Sample stick from the kit, and scrape it firmly across the inside of the subject's cheek 3 – 4 times.
6. Place the sampled end of the Sample stick in its matching labelled Sample Collection tube, approximately 1 cm (0.4"). Move the Collection tube cap to hold the Sample stick in place, and break off the sampled end into the Collection tube. Discard the held end of the broken Sample stick.
7. Add 2 µl of Lysis Agent (yellow cap) to sample.
8. Close cap and vortex sample to mix (automated vortexer, or use a finger to repeatedly 'snap' the tube bottom).
9. Incubate at room temperature for 15 minutes.
10. Transfer the tubes to immerse bottom ends in boiling or near-boiling water for 5 minutes. *A foam float tray can be made by cutting a section from a grocery meat department foam product tray, and using Sample tubes to poke appropriate size holes through the float. Place the samples in the float tray and place, top up, on the surface of a small pan of boiling water. Water can be maintained at a boil e.g. on a hot plate, or can be brought to a boil in a suitable container such as a beaker by a microwave oven, and the samples then floated in this on the bench as the water cools.*
11. Remove the samples from the hot water bath.
12. Add 400 µl 91% isopropanol to each sample, reclose, and let sit at room temperature for 10 minutes.
13. Centrifuge samples at maximum speed (~12,000 rpm) in a microcentrifuge, room temperature, 10 minutes. *Orientation of the tubes in a specific manner, such as cap retainers outward, is recommended during centrifugation as this will deposit the DNA in a known position on the Sample tube and allow easier recovery in subsequent steps.*
14. Carefully pipette off the supernatant and discard. *If the pellet location is assumed based on tube orientation, tip the tube gently and pipette away from that region to avoid dislodging pellet.*
15. Allow pellet to air dry ~5 minutes or until no visible fluids remain in sample.
16. Resuspend sample in 50 µl PCR grade water (white cap). *If pellet is visible, carefully pipette repeatedly until uniformly resuspended.*
17. Sample may be used immediately. For long term storage, -20°C is recommended.

Use as template in molecular assays e.g PCR

Sample yields can vary greatly depending on how effective cheek scraping is and other factors. Average yields as observed by PathoID are approximately 120 ng human genomic DNA per sample. For most PCR applications, these samples may be diluted 1:100 and provide good amplification results.

Quality Control

Each lot of this kit is functionally tested to perform as expected. PCR water included in this kit is reverse osmosis purified, and has been tested at PathoID for lack of observable DNase activity, and lack of amplifiable human gDNA. This water is not necessarily sterile and may contain traces of other DNA types not controlled for.

Reference:

The Quest for the $500 home molecular biology laboratory. Brunstein, J. *Medical Laboratory Observer* **43**(12)26 -29 (2011)

CAUTIONS AND DISCLAIMERS: This kit is provided for **EDUCATIONAL AND ENTERTAINMENT USE ONLY**. Use in any medical diagnostic, clinical, forensic, or agricultural application is not approved, and by use of this product, you agree no such applications will be made. In the event any potentially medically relevant results are suggested through use of this product, the user is recommended to discuss the matter with a qualified medical specialist.

This kit is intended for use by or under the supervision of adults with an understanding of, and in accordance with, basic laboratory safety procedures including but not limited to personal protective equipment (PPE).

LYSIS AGENT MAY IRRITATE EYES. Do not get in eyes. Harmful if swallowed. Keep out of reach of children. **FIRST AID TREATMENT:** contains biodegradable phosphate-free surfactants (anionic and non-ionic) and enzymes. If swallowed, give a glassful of water or milk and and call a Poison Control Center or doctor immediately. Do not induce vomiting. In case of contact with eyes, rinse well with water for 15 minutes.

ALL OTHER KIT CONTENTS non-toxic. All kit products and wastes are non-biohazardous and may be disposed of in routine domestic waste streams in most jurisdictions. If in doubt, please consult local regulations.

PathoID does not warranty this product for any specific end user application other than its intended use when instructions are followed. PathoID accepts no liability, direct or indirect, for the use of this product. In the event of perceived product failure, the end user is recommended to contact PathoID Customer Service at (503) 951-4299 or custserv@pathoid.com with product lot number and description of problem. Sole remedy in the event of a product failure shall be, at PathoID's discretion, either refund for or replacement of the non-performing portion(s) of the product.

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