BECKMAN COULTER
Life Sciences

# DNA extraction from mouthwash samples using GenFind V3 genomics extraction kit

*Researchers working with mouthwash samples and researchers looking for a non-invasive way to collect human cells for genomic analysis would find this supplemental protocol useful.*

*Please reference current GenFind V3 IFU for product information (Part Number: C34880, C34881)*

## Purpose

Mouthwash is a non-invasive way to collect human cells for genomic analysis. As patients are familiar with the use of mouthwash, sampling error is limited compared to similar methods, such as sputum and buccal swabs. Mouthwash samples are taken in large volumes (usually 10 - 30 mL), and cells need to be spun down from the total volume of mouthwash before extraction can begin. This protocol incorporates those steps and optimizes the lysis for mouth cell samples.

## Materials Used

| Material | Part Number | Supplier |
|---|---|---|
| Nuclease-free water (Molecular Grade) | AM9932 | Thermo Fisher Scientific |
| 2 mL 96–well plate | 609681 | Beckman Coulter Life Sciences |
| 7 Bar Magnet for 96-well plate | 771MWZM-1ALT | V&P Scientific |
| 37-degree heat block or water bath | N/A | N/A |

## Protocol

1. **Sample Preparation**
    a. Pellet **10-30 mL** of mouthwash solution by spinning for **10 minutes** at **3,000 x *g***
    b. Remove and discard the supernatant without disrupting the pellet
2. **Lysis**
    a. Add **500 µL** of **Lysis (LBB)**
    b. Add **30 µL** of **Proteinase K (PK)**
    c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    d. Transfer the resuspension to a 2 mL 96-well plate
    e. **Incubate** the plate for **10 minutes** at **37°C**
3. **Bind**
    a. Vortex to fully resuspend the **Bind (BBB)**
    b. Add **300 µL** of **Bind (BBB)** to the plate
    c. **Incubate** the plate for **5 minutes** at **room temperature**
    d. Place the plate on a **magnet** for **15 minutes** (or until supernatant is clear)
    e. Remove and discard the supernatant without disrupting the beads
    f. Remove the plate from the magnet

4. **Wash**
    a. Add **800 µL** of **Wash (WBB)** to plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on a **magnet** for **10 minutes** (or until supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet
    f. Repeat steps 3.a-3.e for a total of **2 washes**
    g. Add **1.6 mL** of **Wash (WBC)** to plate
    h. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    i. Place the plate on a **magnet for 10 minutes** (or until supernatant is clear)
    j. Remove and discard the supernatant without disrupting the beads
    k. Remove the plate from the magnet
    l. Repeat steps 3.g-3.k for a total of **2 washes**

5. **Elute**
    a. Add **40 µL** of **nuclease-free water** to plate
    b. **Incubate** the plate for **2 minutes** at **room temperature**
    c. Place the plate on a **magnet** for **2 minutes** (or until supernatant is clear)
    d. Remove and **save** the supernatant without disrupting the beads

## Example Data

15 mL of mouthwash containing 12.25% alcohol was given to 5 adults. Due to inherent biological variation insert, three samples were pooled to give technical replicates. The other 2 samples were processed individually. A NanoDrop (Thermo Fisher Scientific) was used to assess yield and purity (Table 1, Figure 1). The yields for the technical replicates gave an average yield of 1.8 with a standard deviation of 0.14. The purities for all samples, pooled and individual, gave absorbance ratios sufficient for most downstream applications. Genomic DNA (gDNA) integrity was assessed on an Agilent Genomic DNA Screen Tape (Agilent) (Figure 2). The A260/A230 ratios are sufficient given the high amounts of carbohydrates in saliva samples, which do not affect downstream applications.

| Sample | Concentration (ng/µL) | Yield (µg) | 260/280 | 260/230 |
|---|---|---|---|---|
| pooled | 41.3 | 1.7 | 1.8 | 1.8 |
| pooled | 48.8 | 2.0 | 1.8 | 2.0 |
| pooled | 49.5 | 2.0 | 1.9 | 1.9 |
| a | 90.8 | 3.6 | 1.8 | 1.9 |
| b | 16.3 | 0.7 | 1.7 | 1.5 |

**Table 1.** Concentration, yield and purity of DNA extracted from mouthwash samples. The pooled samples showed very little variation. The large variation seen in the other two samples could be due to biological variation.

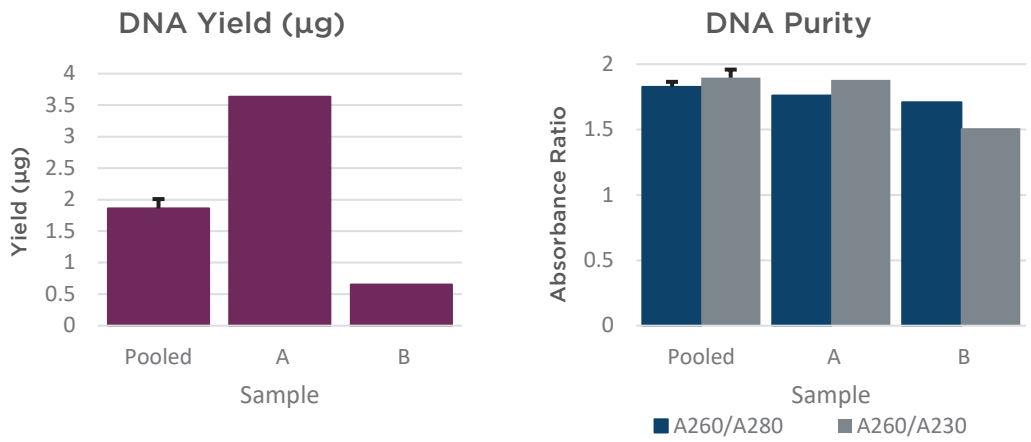


**Figure 1.** (Right) The DNA yield of DNA extracted from mouthwash samples. (Left) The DNA purity of DNA extracted from mouthwash samples. The error bars in the pooled sample are the standard deviation of three technical replicates.

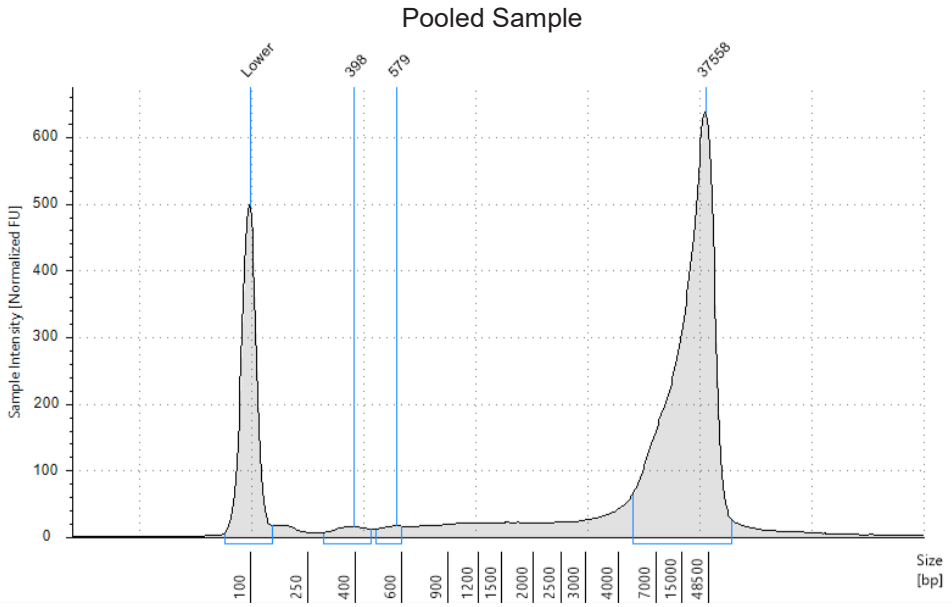


**Figure 2.** An Agilent Genomic DNA Screen Tape of DNA extracted from mouthwash. The electropherogram of one of the pooled samples is shown above. The DIN score was 8.1.


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