BECKMAN COULTER
Life Sciences

gDNA Extraction from Mouthwash with Genfind v2

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.

Please reference the current Genfind v2 protocol for product information
(Part Number A41497, A83078)

Purpose

Mouthwash samples are collected 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 conditions for mouth epithelial cells.

Materials Used

Material	Part Number	Supplier
Genfind v2	A41497, A83078	Beckman Coulter
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
Nuclease-free water (Molecular Grade)	AM9932	ThermoFisher Scientific
1.2 mL 96-well plate	AB1127	ThermoFisher Scientific
Used Scope Outlast Mouthwash	796433742302	Proctor and Gamble
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific

Protocol

1. **Sample Preparation**
   a. Spin 30 mL mouthwash solution down for 10 min at 3000 x g
   b. Decant off supernatant

2. **Lysis**
   a. Resuspend pellet in **400 µL Lysis (LBC) Buffer** and **9 µL Proteinase K**
   b. Transfer **400 µL** of **sample** to 96 well plate
   c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
   d. Incubate for 10 min at 37 ˚C or 1 hr at room temperature (22 ˚C)

3. **Bind**
   a. Vortex to fully resuspend the Bind Buffer (BBB)
   b. Add **300 µL** of **Bind Buffer (BBB)** to the plate
   c. Incubate the plate for **5 min** at **room temperature**
   d. Place the plate on the **magnet** for **15 min** (or until supernatant is clear)
   e. Remove and discard the supernatant without disrupting the beads
   f. Remove the plate from the magnet

4. **Wash 1**
    a. Add **800 µL** of **Wash 1 Buffer (WBB)** to plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on the **magnet** for **10 min** (or until supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet
    f. Repeat step 4 for a total of **2 washes**

5. **Wash 2**
    a. Add **500 µL** of **Wash 2 Buffer (WBC)** to plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on the **magnet** for **6 min** (or until supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet
    f. Repeat step 5 for a total of **2 washes**

6. **Elute**
    a. Add **80 µL** of **nuclease free water** to plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. **Incubate** the plate for **2 min** at **room temperature**
    d. Place the plate on the magnet for **2 min** (or until supernatant is clear)
    e. Remove and **Save** the supernatant without disrupting the beads

Example Data

Donors swished 30 mL of mouthwash for 30s. DNA was extracted from the mouthwash using the protocol listed above. Two different lysis conditions were used: lysis for 1 hr at room temperature and lysis for 10 min at 37˚C. Both conditions were tested in triplicate. DNA concentration was measured by absorbance at λ260. The quality of the DNA was measured using a TapeStation Genomic Tape (Agilent). The absence of PCR inhibitors was confirmed by qPCR with ActB primers (Kapa Sybr Fast qPCR kit, Kapa). 100 ng of DNA was used for the qPCR.

Both lysis conditions resulted in DNA yields of greater than 4.5 µg (Figure 1, Table 1). Variability in yield is due variability between donors. Lysis at 37 ˚C results in slightly higher yields, but both methods provide sufficient DNA for most downstream applications.

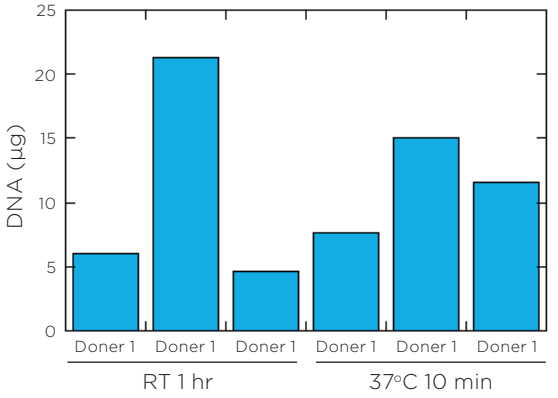

Figure 1. Yield of mouthwash samples under both lysis conditions.

SAMPLE ID	CONC. (ng/µL)	260/ 280	260/ 230	TOTAL YIELD (µg)
RT 1 hr Donor 1	75.4	1.76	1.54	6.0
RT 1 hr Donor 2	265.7	1.93	1.28	21.3
RT 1 hr Donor 3	57.7	1.74	0.23	4.6
37˚C 10 min Donor 4	95.4	1.82	1.42	7.6
37˚C 10 min Donor 5	188.6	1.81	1.13	15.1
37˚C 10 min Donor 6	145	1.81	1.51	11.6

Table 1. Data from individual replicates.

Genomic DNA remained intact with both lysis methods, as seen by peaks at approximately 40,000 bp (Figure 2). Samples also amplified well in a qPCR assay, indicating that PCR inhibitors are not present.

Genfind v2 can be optimized for mouthwash DNA extractions. This method provides sufficient yield for most downstream steps with good integrity. Purity is also good, as seen by qPCR amplification..

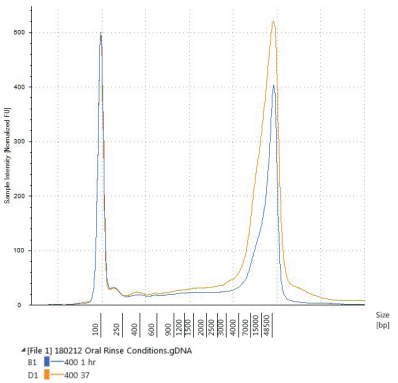

Figure 2. Size of DNA in mouthwash samples lysed for 1 hr at room temperature (blue) and 10 min at 37 ˚C (orange). Samples were run on an Agilent TapeStation genomic tape.

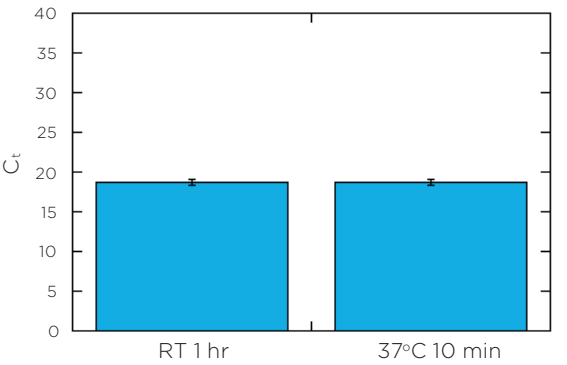

Figure 3. Amplification of gDNA (100 ng) with ActB primers. Critical thresholds (C_t) were consistent with expected values for the DNA concentration amplified. Error bars are the average of three biological replicates.

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