Promega

Product Application

Manual DNA extraction from mouthwash samples

Extract amplifiable DNA from mouthwash samples using the ReliaPrep™ Blood gDNA Miniprep System.

Kit: ReliaPrep™ Blood gDNA Miniprep System (Cat. #A5081)

Analyses: Quantification (by absorbance and by fluorescence)

qPCR amplification

Sample Type(s): Oral rinse with water
Scope Classic® mouthwash samples
Listerine Original®mouthwash samples

Input: 10ml of mouthwash

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM330, available at: www.promega.com/protocols

Materials Required:

- ReliaPrep™ Blood gDNA Miniprep System (Cat. #A5081)
- PBS
- Vortex mixer
- Thermoblock
- Centrifuge
- 1.5ml tube

Protocol:

1. Pre-process mouthwash samples. Samples can be processed fresh or frozen (thaw before processing):
    a. Centrifuge the 10ml mouthwash samples at 10,000 x g for 5 minutes.
    b. Remove supernatant.
    c. Add 1ml of PBS and vortex to resuspend the pellet.
    d. Transfer up to half of the sample (≤500µl) into a 1.5ml tube.
    e. Centrifuge at 2,000 x g for 2 minutes
    f. Remove supernatant.
2. Add 20µl of Proteinase K (PK) Solution to each tube. Vortex.
3. Add 200µl of Cell Lysis Buffer (CLD) to each tube. Vortex for at least 10 seconds.
4. Incubate at 56°C for 10 minutes.
5. Add 250µl of Binding Buffer (BBA) to each tube. Vortex for 10 seconds.
6. Transfer the lysate to the ReliaPrep™ Column. Centrifuge at maximum speed for 1 minute.
7. Remove the collection tube. Place the ReliaPrep™ Column into a new collection tube.
8. Add 500µl of Column Wash Solution (CWD) to the column. Centrifuge at maximum speed for 3 minutes. Discard the flowthrough.
9. Repeat step 8 twice (for a total of three washes).
10. Place the ReliaPrep™ Column into a 1.5ml tube.
11. Add 50µl of Nuclease-Free Water to the column. Centrifuge at maximum speed for 1 minute to elute.

Results:

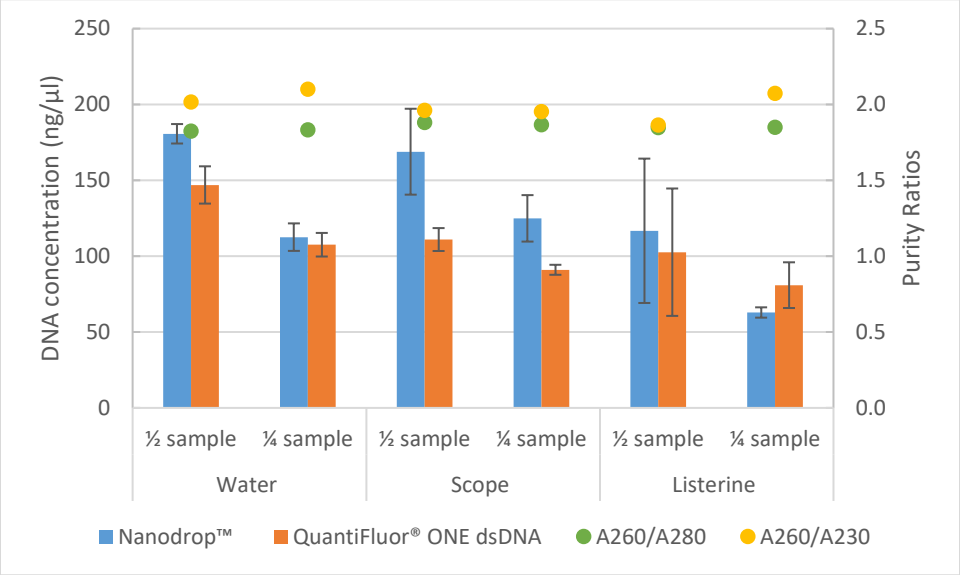

Figure 1. DNA concentration purified from mouthwash samples using the ReliaPrep™ Blood gDNA Miniprep System (Cat. #A5081). DNA was purified in triplicate from ¼ (250µl) or ½ (500µl) of 10ml mouthwash samples using the above protocol. DNA concentration and purity ratios were assessed by NanoDrop™ One and QuantiFluor® ONE dsDNA System (Cat. #E4871). Mean ± STD of n=3 is shown for DNA concentration on the primary axis, and mean absorbance ratios on the secondary axis. After extraction, DNA eluates were amplified using PowerQuant® System (Cat. #PQ5002) and no qPCR inhibition was observed (data not shown).