# Wine/Beverage DNA Extraction Kit

#WINEBEV100 100 preps v1.1 June 2023

Sample: 2mL of wine, beer, cider or other beverage.
Volume may be reduced for active ferments where samples are viscous

Format: Spin column

Elution volume: 100 µL

Storage: dry at room temperature (15 - 25°C)

## Introduction

This kit is designed to purify DNA from bacteria and yeast from a range of beverages, especially those organisms associated with spoilage. The convenient spin column means the process can be completed within 40 minutes, without the use of hazardous chemicals.

## Contents of kit

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|---|---|
| GT Buffer | 30 mL |
| Proteinase K | 11 mg x 2 (add 1.1 mL sterile water or elution buffer) |
| GBT Buffer | 40 mL |
| W1 Buffer | 45 mL |
| Wash Buffer | 25 mL (Add 100 mL reagent grade absolute ethanol) |
| GS columns (bag) | 100 |
| 2ml collection tubes | 200 |

## Protocol

IMPORTANT NOTES BEFORE USE!

• Pulse centrifuge a proteinase K tube and add 1.1 mL elution buffer to the enzyme. Vortex well and leave for 5 minutes before use. Enzyme may be stored at 4 ˚C for up to a month, otherwise store at -20 ˚C.

• Add 100 mL absolute ethanol to the Wash Buffer bottle prior to initial use. Mix and cap tightly.

• Additional requirements: absolute ethanol, microcentrifuge tubes (DNase and RNase-free), pipettes. sterile transfer pipettes.

**DNA extraction**

- Transfer 2 mL of wine/beer/cider other beverage to a 2 mL microcentrifuge tube. *Reduce this volume to 1 mL for active ferments or high yeast samples (e.g. lees).*
- Centrifuge the samples at 12,000 – 15,000 x g for 5 minutes.
- Using a disposable 2mL transfer pipette, remove the supernatant and discard.
- Add 200 µL of **GT buffer** to the pellet along with 20 µL reconstituted proteinase K (see notes before use above).
- Vortex well and rake along a microcentrifuge rack to break up the pellet.
- Incubate at 60 ˚C for ≥10 minutes (see notes below)
- Pulse spin to collect the contents at the bottom of the tube and add 200 µL of **GBT buffer** to the samples.
- Vortex and incubate at 60 ˚C for ≥10 minutes.
  During this incubation, add ~110 µL of **Elution Buffer** to a microcentrifuge tube and incubate at 60 ˚C until required at the last step.
- Pulse spin to collect the contents at the bottom of the tube and add 200 µL of absolute ethanol to the samples.
- Vortex samples well.
  *If cellular material is present (e.g. lees material) then centrifuge the samples at 12,000 – 15,000 x g for 30 seconds.*
- Assemble a GS column into a 2ml collection tube and transfer the supernatant mixture from the previous step into the top of the GS column
- Centrifuge the column assembly (column plus collection tube) at 12,000 – 15,000 x g for 1 minute.
- Transfer the GS column to a new collection tube and add 400 µL **W1 Buffer** to the column.

- Centrifuge the column assembly (column plus collection tube) at 12,000 – 15,000 x g for 30 seconds.

- Discard the contents of the collection tube and reassemble the column into the tube. Add 600 µL **Wash Buffer** (with ethanol added – see notes before use above).

- Centrifuge the column assembly (column plus collection tube) at 12,000 – 15,000 x g for 30 seconds and discard the contents of the collection tube as previously.

**DNA extraction *continued***

- Reassemble GS column and collection tube then add 200 µL Wash Buffer to GS column. Centrifuge at 12,000 – 15,000 x g for **1 minute**.

- Transfer the GS column to a microcentrifuge tube and slowly add 100 µL pre-heated Elution Buffer to the centre of the matrix in the column.

- Leave column assemblies in rack on the bench for 3-5 minutes.
  *This step allows the column matrix to absorb the heated elution buffer and release the DNA.*

- Centrifuge the column/tube assembly at 12,000 – 15,000 x g for 30 seconds to elute the DNA from the column.
  *Place the tubes in the microcentrifuge so that the caps are facing one direction (opposite to the rotation direction – this prevents cap breakage).*

- The eluted DNA is now in the microcentrifuge tube and is ready for PCR or qPCR.

DNA may be stored at 4 ˚C for up to 5 days or store at -20 ˚C for long term retention.

**Notes**

1. The incubation after adding GT Buffer may be extended to 20-30 minutes, if risk of evaporation is minimal.

2. A convenient stopping point is after the addition of the GBT buffer or the absolute ethanol to the sample/GT/GBT mixture. Samples may be left at room temperature overnight.

3. Rather than empty contents of collection tubes, GS column may be transferred to a new collection tube (additional tubes available from dnature or other suppliers).