7. Add 500 μL Buffer GW2 to a adsorption column (please check whether 100% ethanol has been added before use), centrifuge at 12,000 rpm for 1 min, dump the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

   Note:

   Repeat Step 7 if you need to further improve DNA purity.

8. Centrifuge at 12,000 rpm for 2 min and discard the waste solution in the collection Tube.Leave the adsorption column at room temperature for a few minutes to dry thoroughly.

   Note:

   The purpose of this step is to remove residual ethanol from the adsorption column, which will affect subsequent enzymatic reactions (enzyme digestion, PCR, etc.).

9. The adsorption column is placed in a new centrifuge tube (self-provided), and 50-200 μL Buffer GE or RNase-Free water is added to the middle part of the adsorption column. The adsorption column is placed at room temperature for 2-5 min and centrifuged at 12,000 rpm for 1 min. DNA solution is collected and stored at -20°C.

   Note:

   1) If the downstream experiment is sensitive to pH or EDTA, it can be eluted with RNase-Free water. The pH value of eluent has a great influence on the elution efficiency. If water is used as the eluent, the pH value should be within 7.0-8.5 (NaOH can be used to adjust the pH value of water to this range). When pH value is lower than 7.0, the elution efficiency is not high.

   2) Buffer GE is preheated in 65-70°C water bath and incubated at room temperature for 5 minutes before centrifugation to increase the yield.

   3) Because DNA stored in water will be affected by acid hydrolysis, it is recommended to eluate with Buffer GE and store at -20°C if long-term preservation is required.

Version: 12/2023

Saliva Genomic DNA Kit

Cat. No. : CW2655S (50 preps)
CW2655M (200 preps)
Storage Condition : Room temperature (15-25°C)

Components

Component	CW2655S 50 preps	CW2655M 200 preps
Buffer GL	25 mL	100 mL
Buffer GW1 (concentrate)	13 mL	52 mL
Buffer GW2 (concentrate)	15 mL	50 mL
Buffer GE	15 mL	60 mL
Proteinase K	2×1.25 mL	2×5 mL
Spin Columns DM with Collection Tubes	50	200

Introduction

This kit is suitable for the extraction of genomic DNA from fresh saliva or saliva/preservation mixtures. The purification process of this product does not need toxic solvents such as phenol or chloroform, and does not need ethanol precipitation. The optimized buffer system enables the DNA to be efficiently and specifically bound to the silicon matrix centrifugal adsorption column. The inhibitors of PCR and other enzymatic reactions can be effectively removed through two washing steps. Finally, the DNA can be eluted with low salt buffer solution or water to obtain high purity DNA. The purified DNA can be directly used in enzyme digestion, PCR, Real-Time PCR, library construction, Southern Blot, molecular labeling and other downstream experiments.

Reagents to be Supplied by user

100% ethanol

Precautions

1. Repeated freezing and thawing of samples should be avoided, otherwise, the extracted DNA fragments will be small and the extracted amount will decrease.
2. 100% ethanol should be added to Buffer GW1 and Buffer GW2 according to the instructions of reagent bottle label before the first use.
3. Check Buffer GL for crystallization or precipitation before use, and if crystallization or precipitation occurs, redissolve Buffer GL in a 56°C water bath.
4. If the downstream experiments are sensitive to RNA contamination, 4 μL DNase-Free RNaseA (100 mg/mL) can be added in step 3. RNaseA is not provided in this kit, and if needed, it can be ordered separately from our company (Cat. No.: CW0601).
5. For prolonged storage of saliva DNA at room temperature, our Saliva DNA Storage Tube (Cat. No.: CW2667) is recommended.

Protocol

1. Add saliva sample or saliva/preservation solution mixture 400 μL.

   Note:

   1) The saliva mixture added to the preservation solution should be bathed in water at 50℃ for 1 hour or in an empty temperature tank at 50℃ for 2 hours before extraction.

   2) If the sample volume needs to be increased, the volume of Proteinase K, Buffer GL and anhydrous ethanol in Steps 2-4 should be multiplied. The liquid in step 5 can be transferred several times.
2. Add 40 μL Proteinase K.
3. Add 400 μL Buffer GL, vortex shock thoroughly mix, 56°C water bath for 15-30 min.

   Note:

   To remove RNA, add 4 μL of 100 mg/mL RNase A solution (Cat. No.: CW0601), swirl for 15 s and leave at room temperature for 2 min after the above steps.
4. Centrifuge briefly to remove water beads from the inner wall of the tube cover. Add 400 μL anhydrous ethanol and mix thoroughly in vortex. Temporary centrifugation.

   Note:

   1) Mix in vortex immediately after adding Buffer GL and anhydrous ethanol.

   2) White precipitation may be produced after the addition of Buffer GL and anhydrous ethanol, which will not affect the subsequent experiment.

   3) Sol-like products may be formed after GL and anhydrous ethanol, in which case violent shock or vortex treatment is recommended.
5. All the solution obtained in the previous step is added into the Spin Column DM which has been loaded into the Collection Tube. If the solution cannot be added at one time, it can be transferred several times. Centrifuge at 12,000 rpm (~ 13,400 ×g) for 1 min, dump the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
6. Add 500 μL Buffer GW1 to a adsorption column (please check whether 100% ethanol has been added before use), centrifuge at 12,000 rpm for 1 min, dump the waste liquid in the collection tube, and put the adsorption column back into the collection tube.