Version: 12/2023

# NuClean Magbead FFPE DNA Kit

**Cat. No. :** CW2558S (32 preps)
CW2558M (96 preps)

**Storage Conditions:** Room temperature (15-30°C)

## Components

| Component | CW2558S 32 preps | CW2558S 96 preps |
|---|---|---|
| Buffer DS | 10 mL | 25 mL |
| Buffer GTL | 10 mL | 30 mL |
| Buffer GL | 10 mL | 30 mL |
| Buffer GW1 (concentrate) | 30 mL | 80 mL |
| Buffer GW2 (concentrate) | 20 mL | 50 mL |
| Buffer TE | 10 mL | 30 mL |
| Proteinase K | 2×20 mg | 4×25 mg |
| Proteinase K Storage Buffer | 2×1 mL | 4×1.25 mL |
| RNase A (100 mg/mL) | 0.1 mL | 0.3 mL |
| Magbeads PN | 1 mL | 2×1 mL |

This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

## Introduction

The kit provides a simple, rapid and efficient method for extracting DNA from paraffin-embedded tissues. Non-toxic dewaxing agent can be used to remove paraffin during the experiment to reduce the harm to the experimenter. After tissue lysis, DNA is bound to the surface of silica-based encapsulated magbeads. After rinsing, high purity DNA is eluted in EB or deionised water. The purified DNA can be used directly for PCR, Real-time PCR, SNP genotyping, STR genotyping, next-generation sequencing and pharmacogenomics research. The yield of DNA and fragment size are highly dependent on the type of sample, storage conditions and storage time.

## Reagents to Be Supplied by User

1. Automatic Nucleic Acid Extractor
2. 96 DW Plate ; 8 channel Comb
3. 100% ethanol; Isopropanol

## Precautions

1. After obtaining the sample, fix the sample as soon as possible, and the fixation time should be 14-24 hours. If the time is too long, the genome will be broken and the downstream experiments will be affected. If the formaldehyde fixation time is too long or the sample is stored for a long time (>1 year), the integrity of the DNA is easily damaged, and long fragments cannot be amplified.
2. Make sure that the sample before embedding is completely dehydrated, and the residual formalin will inhibit the effect of Proteinase K.
3. Add 1 mL Proteinase K Storage Buffer to Proteinase K to dissolve it, and store at -20℃. Prolonged storage of formulated Proteinase K at room temperature will affect its activity.
4. Before the first use, add 100% ethanol to Buffer GW1 and Buffer GW2 according to the instructions on the reagent bottle label.
5. Before use, please check whether Buffer GTL, Buffer GL and Buffer DS are crystallized or precipitated. If there is crystallisation or precipitation, please re-dissolve Buffer GTL, Buffer GL and Buffer DS in a water bath at 56°C.

## Match with CWE2100 automatic nucleic acid extractor

1. Add the appropriate reagents to the 96 DW Plate according to the table below.

| Position | Reagent |
|---|---|
| Colume 1&7 | Lysate: all<br>Buffer GL: 200 μL<br>Isopropanol: 300 μL<br>Magbeads PN: 20 μL |
| Colume 2&8 | Buffer GW1: 750 μL |
| Colume 3&9 | Buffer GW1: 750 μL |
| Colume 4&10 | Buffer GW2: 750 μL |
| Colume 5&11 | Buffer GW2: 750 μL |
| Colume 6&12 | Buffer TE: 70 μL |

**Note: In columns 1 & 7, Magbeads PN can be added after mixing with isopropanol in the proportions shown in the table.**

2. Put the 96 DW plate and 8 channel Comb into CWE2100. Run the FFPE DNA program.
3. After about 30 minutes, the program is finished. Take out the 96 DW plate and 8 channel Comb. Transfer the elution products from column 6&12 to centrifuge tubes and store at -20°C.

Attachment: FFPE DNA program

| Position | Release magbeads | Name | Wait time | Mix time | Mix speed | Number of Cycles | Magnetic time | Volume (μL) | Temperature |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Yes | Mix | 0 | 2 min<br>3 min | fast<br>medium | 1 | Once for 5 seconds, 3 times | 700 | Room temperature |
| 2 | Yes | Mix | 0 | 2 min | fast | 1 | Once for 1 second, twice | 750 | Room temperature |
| 3 | Yes | Mix | 0 | 2 min | fast | 1 | Once for 1 second, twice | 750 | Room temperature |
| 4 | Yes | Mix | 0 | 2 min | fast | 1 | Once for 1 second, twice | 750 | Room temperature |
| 5 | Yes | Mix | 0 | 2 min | fast | 1 | Once for 1 second, twice | 750 | Room temperature |
| 5 | No | Dry | 5 min | 0 | 0 | 0 | 0 | 0 | Room temperature |
| 6 | Yes | Elute | 0 | 2 min<br>2 min | medium<br>fast | 2 | Once for 10 seconds, 5 times | 70 | 60°C |
| 4 | Yes | Release | 0 | 10 s | fast | 0 | 0 | 750 | Room temperature |



6. If downstream experiments are sensitive to RNA contamination, 2 μL of DNase-Free RNase A (100 mg/mL) can be added before adding Buffer GL. RNase A is recommended to be stored at -20°C if not used for a long time.
7. Before starting the experiment, preheat the water bath or constant temperature mixer to 56°C.
8. Magbeads PN should not be frozen or centrifuged at high speed as this may cause irreversible damage to Magbeads PN. Magbeads PN should be shaken well and mixed well each time before use.

## Protocol

### i Paraffin-embedded samples

1. Trim off excess paraffin from the tissue block to expose the tissue, and cut into 5-10 μm slices.
2. Take about 1×1 $cm^2$ slices (about 3-8 slices in total) and put them in a centrifuge tube (self-provided), add 160 μL Buffer DS, and vortex for 10 s. Add 180 μL of Buffer GTL and 20 μL of Proteinase K, and vortex for 10 s. Centrifuge at 12,000 rpm for 1 min.

   **Note: 1) If the sample surface is exposed to air, discard the initial 2-3 slices.**

   **2) DS will solidify when it is below 18°C. If it solidifies, it will not affect the following experiment.**
3. Incubate at 56°C for 1 hour until the sample is completely dissolved. Incubate at 90°C for 1 hour. Centrifuge at 12,000 rpm at 25°C for 1 min. Use a pipette to carefully pipette the lower aqueous phase (about 180 μL) along the tube wall into a new centrifuge tube, avoiding the suction of the sediment at the bottom of the tube and the upper wax liquid.

   **Note: 1) After incubation at 56°C, the sample can be left at room temperature until the water bath or dry bath reaches 90°C and then incubated at 90°C.**

   **2) Optional step: Add 7 μL UNG (1 U/μL) at 50°C for 5min without shaking. The purpose of this step is to reduce the C>T | G>A transition (artificial mutation) at low frequency, and at the same time effectively retain the real mutation, so as to minimize the risk of false positive. UNG (Cat. No. : CW0951S) is not supplied with this kit, but can be ordered separately from us if required.**

4. Optional step: To remove RNA, add 2 μL of RNase A (100 mg/mL), shake and mix well, and leave at room temperature for 2 minutes.
5. Add 20 μL of Proteinase K and incubate at 65°C for 15 min at 450 rpm to form Lysate.

**ii Samples in fixative such as formalin:**

1. Take approximately 20 mg of sample, cut into small pieces, place in a centrifuge tube and add 500 μL of 10 mM PBS (pH 7.4). Vortex shake and centrifuge at 12,000 rpm (~13,400×g) for 1 min, discard supernatant and repeat 3 times.
2. Add 180 μL of Buffer GTL and 20 μL of Proteinase K to the above tubes and mix well by vortex shaking.
3. Incubate at 56°C for 1 hour until the sample is completely dissolved. Incubate at 90°C for 1 hour. Sample left to stand at room temperature for 30 s. Centrifuge at 12,000 rpm at 25°C for 1 min. Use a pipette to carefully pipette the lower aqueous phase (about 180 μL) along the tube wall into a new centrifuge tube, avoiding the suction of the sediment at the bottom of the tube and the upper wax liquid.

   **Note: 1) After incubation at 56°C, the sample can be left at room temperature until the water bath or dry bath reaches 90°C and then incubated at 90°C.**

   **2) Optional step: Add 7 μL UNG (1 U/μL) at 50°C for 5min without shaking. The purpose of this step is to reduce the C>T | G>A transition (artificial mutation) at low frequency, and at the same time effectively retain the real mutation, so as to minimize the risk of false positive. UNG (Cat. No. : CW0951S) is not supplied with this kit, but can be ordered separately from us if required.**
4. Optional step: To remove RNA, add 2 μL of RNase A (100 mg/mL), shake and mix well, and leave at room temperature for 2 minutes.
5. Add 20 μL of Proteinase K and incubate at 65°C for 15 min at 450 rpm to form Lysate.



## Manual operation steps

1. Add 200 μL of Buffer GL to Lysate and vortex shake to mix well.
2. Add 300 μL of isopropanol and 20 μL of magbeads to the centrifuge tube and vortex shake for 5 s, then fix the tube on a thermostatic mixer at 25°C and 1600 rpm for 10 min.
3. Centrifuge tube is fixed on a magnetic stand and left to stand for 1 min. Discard the solution.
4. Add 750 μL Buffer GW1 (please check whether 100% ethanol has been added before use) to the centrifuge tube, vortex for 5 s, and then put it on a thermostatic mixer at 25°C and 1600 rpm for 2 min.
5. Centrifuge tube is fixed on a magnetic stand and left to stand for 1 min. Discard the solution.
6. Repeat steps 4-5.
7. Add 750 μL of Buffer GW2 (check that 100% ethanol has been added before use) to the centrifuge tube, vortex for 5 s and place on a thermostatic mixer at 25°C and 1600 rpm for 2 min.
8. Centrifuge tube is fixed on a magnetic stand and left to stand for 1 min. Discard the solution.
9. Repeat steps 7-8.
10. Centrifuge briefly, then place the centrifuge tube back on the magnetic stand and further remove the bottom solution with a pipette. Place the centrifuge tube on a magnetic stand for 5-10 min to fully evaporate the ethanol.
11. Add 50-200 μL Buffer TE to the centrifuge tube, vortex and mix, and then place the centrifuge tube on a thermostatic mixer at 25°C and 1600 rpm for 10 min.
12. Place the centrifuge tube on a magnetic stand for 2 min, then transfer the eluate to a new centrifuge tube and store at -20°C.