Protocol for genomic DNA extraction from FFPE Tissue (10 Reactions)

Introduction:

The gDNA extraction from FFPE Tissue Kit is optimised for purification of DNA from FFPE tissue sections. The kit combines the selective binding properties of a Magnetic bead based technology with the usage of safer and effective deparaffinization buffer and a flexible elution volume range between 50ul and 100 µl.

Specially optimised lysis conditions allow genomic DNA to be efficiently purified from FFPE tissue sections with overnight incubation. Incubation at an elevated temperature after proteinase K digestion removes formalin crosslinking of the released DNA, improving yields, as well as DNA performance in downstream assays.

Note that DNA isolated from FFPE samples is usually of lower molecular weight than DNA from fresh or frozen samples. The degree of fragmentation depends on the type and age of the sample and the conditions used for fixation.

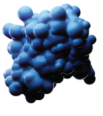
For higher yield and better purities of extracted DNA, we recommend the users to follow all the steps given below without any deviation.

Buffers in the kit:

Sl no.	Buffer	Volume provided in the kit for 10 reactions (ml)	Volume needed per reaction (ml)
1	Deparaffinization buffer	3.0	0.3
2	Tissue Lysis Buffer	2.0	0.2
3	Proteinase K	10 mg	-20°C (upon reconstitution)
4	Proteinase K diluent	0.5	0.03 (30ul)
5	LBB	5	0.5
6	Cambead solution	0.5	0.05 (50ul)
7	Wash Buffer I	4.42	0.5
8	Wash buffer II	3	0.5
9	Elution buffer	5	0.05 (50ul)

Preparation of working solutions

1. **PROTEINASE K solution** : Reconstitute the lyophilized **Proteinase K** powder by adding the entire 0.5 mL of Proteinase K diluent to the Proteinase K tube directly. After reconstitution the Proteinase K should be stored at -20°C.
2. **Wash Buffer I** - Add 3.58 mL of 100% Ethanol to Wash I to make up the volume upto 8 mL



3. **Wash Buffer II** - Add 5mL of 100% Ethanol to Wash II to make up the volume upto 8 mL
4. **Wash Buffer III** is 80% Ethanol which can be prepared before processing the samples.
5. Add the required amount of ethanol to the Wash buffers I and II before use.

Requirements (NOT PROVIDED WITH THE KIT):

- 1.5ml Microcentrifuge tubes
- Magstands
- Pipettes
- Microtips
- 100% Ethanol
- Thermomixer / Dry bath incubator
- Vortex mixer
- Centrifuge

Procedure

A. Deparaffinization and Lysis of sample

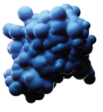
1. Take 3- 4 curls of 10µm FFPE sample and place in a 1.5mL microcentrifuge tube (MCT)
2. Centrifuge the MCT at maximum RPM for 3 minutes to settle the curls as pellet
3. Add 0.3ml (300ul) of deparaffinization buffer into the tube containing the sample and incubate at 80℃ for 2 minutes.
4. Add 200ul of TL buffer and 30ul of reconstituted Proteinase K to the samples and vortex the sample tubes for 10 secs.
5. Centrifuge the sample tubes at 10,000 rpm for 20 seconds. This step will separate two distinct layers of samples and oil in the tube.
6. Incubate the sample tubes at 56℃ on a Thermomixer at 1000 rpm for 16 hours.
   NOTE: For 16 hours incubation step, a Thermomixer is recommended for effective lysis of the sample. But in case of unavailability, a Heat block can also be used but ensure that the samples should be vortexed in between of the incubation as much as possible.

B. Decrosslinking of sample:

1. Post Incubation for 16 hours, incubate the samples furthermore for 1 hour at 90℃ on a Dry bath or Heat Block.
   NOTE: No mixing,vortexing or agitating of samples should be performed during this incubation. They should be left static on the heat block.
2. After the incubation is over, cool the samples for 1-2 minutes

C. Extraction of DNA:

- Using a 200ul Pipette, carefully pipette out the aqueous layer or the bottom layer from the sample tube and transfer that to a new Microcentrifuge tube. This layer will be used for DNA extraction.
  - **NOTE: Ensure that the mineral oil layer or the top layer remains undisturbed.**
- Add 500 µL of LBB Buffer to the sample and 50 µL of CamBeads to the microcentrifuge tube. Incubate the samples for 10 minutes at Room Temperature.



(**NOTE:** Vortex the tubes for 30 seconds after every 2- 3 minutes in a time period of 10 minutes)

- Place the tubes on a magstand. Wait for the magnetite Cambeads to settle on the wall of the microcentrifuge tube. Discard the lysate.
- Add 500 µL of Wash Buffer I to the beads and remove the tubes from the stand. Let the samples sit in the wash buffer for 2 minutes. Vortex the tube for 15 seconds to wash the beads and carefully transfer the entire solution to a new microcentrifuge tube .
- Place the tubes on a magstand. Wait for the magnetite Cambeads to settle on the wall of the microcentrifuge tube. Discard the supernatant.
- Add 500 µL of Wash Buffer II to the beads and remove the tubes from the stand. Let the samples sit in the wash buffer for 2 minutes. Vortex the tube for 15 seconds to wash the beads and carefully transfer the entire solution to a new microcentrifuge tube .
- Place the tubes on a magstand. Wait for the magnetite Cambeads to settle on the wall of the microcentrifuge tube. Discard the supernatant.
- Add 300 µL of Wash Buffer III (80% Ethanol) to the beads and remove the tubes from the stand. Let the samples sit in the wash buffer for 2 minutes. Vortex the tube for 15 seconds to wash the beads and carefully transfer the entire solution to a new microcentrifuge tube .
- Place the tubes on a magstand. Wait for the magnetite Cambeads to settle on the wall of the microcentrifuge tube. Discard the supernatant.
- Air dry the beads at room temperature for at least 5-10 minutes. **Ensure no remnants of Ethanol are found in the samples.**
- While the beads are drying, incubate the elution buffer at 60-70℃ for 10-15 mins.
- Remove the tube from the magstand. When all traces of ethanol are removed and the beads are dry, add 50 µL of pre-warmed elution buffer to the tube.
- Place the tubes on a thermomixer at 56℃ for 5 minutes for efficient elution.
    - **NOTE:** A heated elution step is recommended for obtaining higher yield of extracted DNA.
- Transfer the tube to the magstand. Once the beads have settled against the wall of the microcentrifuge tube, collect the eluted DNA in a fresh DNAse- RNAse- free microcentrifuge tube and store it at -20°C.