Standard Operation Procedure

Protocol for genomic DNA extraction from FFPE Tissue withKingfisher Flex

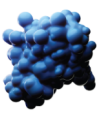
Introduction:

Cambrian gDNA extraction from FFPE Tissue Kit is optimized 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 optimized 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.



Kit contents:

Contents	Volume provided in the kit (250 reactions)	Volume used per reaction (ml)	Storage
Deparaffinization buffer	75 mL	0.3	Room temperature
Lysis Buffer (TLB)	50 mL	0.2	Room temperature
Proteinase K	150mg	.....	-20°C (upon reconstitution)
Proteinase K diluent (PKD)	7.5mL	0.03 (30ul)	Room temperature
Lysis/Binding Buffer	125 mL	0.5	Room temperature
CamBeads solution	12.5 mL	0.05 (50ul)	Room temperature
Wash Buffer I	69 mL	0.5	Room temperature
Wash Buffer II	46.25 mL	0.5	Room temperature
Elution Buffer	12.5 mL	0.0.5 (50ul)	Room temperature
96-well Tip Comb	3	.....	Room temperature
96-well Deep well plate	12	.....	Room temperature
Elution Plate	3	.....	Room temperature

How to start

Items required by the user:

1. ThermoFisher Kingfisher Flex system

Requirements (NOT PROVIDED WITH THE KIT):

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

Preparation of working solutions

1. **PROTEINASE K solution** : Reconstitute the lyophilized **Proteinase K** powder by adding the entire 7.5mL 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 56 mL of 100% Ethanol to Wash I to make up the volume upto 100 mL
3. **Wash Buffer II** - Add 78.75 mL of 100% Ethanol to Wash II to make up the volume upto 100 mL
4. **Wash Buffer III** is 80% Ethanol which can be prepared before processing the samples.

Pre-processing of the FFPE sample before automated extraction

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 pellets.
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 or overnight.
   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

Protocol for genomic DNA extraction

A. Setting up the plates on the Kingfisher Flex platform

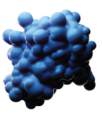
1. Fill the appropriate volume of buffers into the deep-well plate according to the specified volumes in the below-mentioned table and keep them ready.
2. Take a 200ul Pipette and set it at 190ul. **Carefully pipette out 190ul of the aqueous layer or the bottom layer** from the overnight incubated sample and transfer that to the sample plate. **Ensure that the top layer remains undisturbed.**

NOTE:

- Ensure that only the bottom layer is aspirated and no remnants of the top layer is observed.
- While aspirating the bottom layer, do not pipette out the entire aqueous layer, that may lead to top layer contamination.
- Do not change the order of addition of the buffers into the wells of the deep-well plate.
- Vortex the magbead/lysis-binding buffer mixture thoroughly before adding it to the wells of the Sample plate.

Plate Index	Plate Position	Buffer	Volume	Plate type
Sample Plate	1	• Lysis/Binding • Cambeads • Pre-processed FFPE sample	• 500 µL • 50 µL • 190 µL	96-well deep well V bottom plate
Wash I Plate	2	Wash Buffer I	500 µL	96-well deep well V bottom plate
Wash II Plate	3	Wash Buffer II	500 µL	96-well deep well V bottom plate
Wash III Plate	4	Wash Buffer III	300 µL	96-well deep well V bottom plate
Elution Plate	5	Elution Buffer	50 µL	96-well deep well V bottom plate
96-well Tip comb	6	Place 96-well tip comb into a standard 96-well deep well plate		

3. Ensure that the program "**KFF_FFPE DNA extraction.bdz**" is downloaded and installed in the machine.



B. Loading the plates into the Kingfisher Flex extraction platform

1. Select the installed program "**KFF_FFPE DNA extraction.bdz** and run the program.
2. Once prompted by the instrument, load the plates onto their appropriate positions on the extraction platform and start the extraction process.
3. After the extraction process is complete, collect the eluates from the elution plate and store the eluted DNA at -20°C.