Sample Type

Source	Input sample	Expected Yield (µg)
Fresh Tissue	25 mg	5-30
Sputum	200-400 µL	10-30
Pus	200-400 µL	10-30
Amniotic Fluid	1 mL	5-15
CVS	10-25 mg	5-20
Mouse Tail	0.2-0.8 cm	20-40
Mouse Spleen	20-25 mg	10-20
Mouse Kidney	25 mg	10-40
Mouse Liver	25 mg	10-40

Sample Pre-processing

Sputum and pus:

1. Take 200-400 µL of sputum sample in 500 µL of saline solution and centrifuge at 8000 x g for 5 minutes and remove the supernatant.
2. Resuspend the pellet in 500 µL of saline solution and centrifuge at 8000 x g for 5 minutes and remove the supernatant.
3. Continue from Step 1 in the Extraction Protocol.

Amniotic Fluid:

1. Take 10 mL of Amniotic Fluid sample and Centrifuge at 8000 x g for 10 minutes and remove the supernatant.
2. If the clear pellet has not formed, Repeat the Centrifugation and remove the supernatant.
3. Continue from Step 1 in the Extraction Protocol.

Recommended amount of sample for starting:

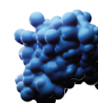
The DNA yield increases linearly with the amount of sample material used, 10-25mg of the sample is recommended as starting material.

Storage of Tissue samples

Fresh sample material or the samples that are immediately frozen and stored at -90°C to -20°C will yield the best results.

Repeated freeze-thaw of the sample should be avoided as it leads to poor DNA quality. Using poor-quality starting material will also result in reduced yield.

DNA yield and quality will slowly decrease due to the prolonged storage of tissue samples under these conditions.



Standard Operation Procedure

Genomic DNA Extraction From Tissue Using Kingfisher Flex

Kit Contents

CONTENT	QUANTITY (1 x 50 RXN.)	STORAGE
TL Buffer (TL)	10 mL	Room temperature
BL Buffer (BL)	10 mL	Room temperature
Proteinase K (lyophilized) (PK)	30 mg	-20°C (upon reconstitution)
Proteinase K Diluent (PKD)	1.5 mL	Room temperature
Lysis-Binding Buffer (LBB)	25 mL	Room temperature
CamBeads (CB)	1 mL	Room temperature
Wash Buffer I (WB1)	16.56 mL	Room temperature
Wash Buffer II (WB2)	11.1 mL	Room temperature
Elution Buffer (EB)	5 mL	Room temperature
96-well Tip Comb	1	Room temperature
96-well Deep well plate	5	Room temperature
Elution Plate	1	Room temperature

How to start

Items required by the user:

1. Kingfisher Flex extraction system.
2. TL Buffer may precipitate during storage. If necessary, warm the buffer container at 70°C until the precipitate fully dissolves.
3. Set an incubator or water bath to 56°C.

Additional buffers to be prepared by the user:

● RNase A

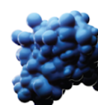
● 80% Ethanol

Preparation of Working Solutions

1. **PROTEINASE K solution**: Reconstitute the lyophilized **Proteinase K** powder by adding 0.5 mL of Proteinase K diluent. After reconstitution, the Proteinase K is stored at -20°C.
2. **Wash Buffer** I: To the Wash Buffer I concentrate, add 3.58 mL of 100% ethanol and shake the bottle thoroughly before use.
3. **Wash Buffer II**: To the Wash Buffer II concentrate, add 5 mL of 100% ethanol and shake the bottle thoroughly before use.

Sample Lysis

1. **Aliquot 25 mg** of animal or human tissue into small pieces. Place the samples into microcentrifuge tubes and proceed with step 2.
    - *Grinding the tissues using a micro pestle into small pieces is recommended for rapid/efficient tissue lysis.*
    - *Sample lysis time may be reduced by disrupting the sample using liquid nitrogen, a bead mill, or treated with a mechanical homogenizer.*
    - *Ensure the correct amount of starting material is used.*
2. Add **200 µL TL Buffer** and **25 µL proteinase K** to the microcentrifuge tube containing the sample solution. Vortex vigorously and make sure the samples are completely submerged. Incubate the microcentrifuge tube at **56°C for at least 3h** or until the samples are completely lysed. Vortexing for **30 seconds every 20 minutes** will help to speed up the lysis process.
    - *Samples may be incubated overnight.*
    - *Once the lysis is complete, centrifuge the samples at 11000x g for 5 minutes and proceed to step 3 with the supernatant.*
    - *Additional PL1 may be added to reduce lysis time, the whole lysate should be taken to proceed with the run.*
    - *If RNA-free DNA is needed, the samples are treated with 10ul of Rnase and incubated at RT for 10 min.*
3. Add 200 µL of **BL Buffer** to the lysate and vortex the mixture vigorously (1 min).



Protocol for genomic DNA extraction from tissue

A. Setting up the plates on the Kingfisher Flex platform

1. Fill the appropriate volume of buffers into the deep-well plates according to the specified volumes in the below-mentioned table and keep them ready.

PLATE INDEX	PLATE POSITION	BUFFER	VOLUME	PLATE TYPE
Sample	1	Tissue Lysate	425 µL	96-well deep well v-bottom plate
		CamBeads	20 µL	
		LBB Buffer	500 µL	
Wash I Plate	2	Wash Buffer I	300 µL	96-well deep well v-bottom plate
Wash II Plate	3	Wash Buffer II	300 µL	96-well deep well v-bottom plate
Wash III Plate	4	Wash Buffer III 80% Ethanol	300 µL	96-well deep well v-bottom plate
Elution Plate	5	Elution Buffer	100 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		

2. Ensure the program **"KFF_AF_Protocol.bdz"** is downloaded and installed on the machine.

B. Loading the plates into the Kingfisher Flex extraction platform

1. After the sample row preparation, select the installed program **"KFF_AF_Protocol.bdz"** and run the program.
2. Once prompted by the instrument, load the prepared 96 deep-well plates onto their appropriate position on the extraction platform.
3. After the extraction process, collect the eluates from the elution plate and store the eluted DNA at -20°C.