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 supernatant.
3. Continue from Step 1 in the Extraction Protocol.

Recommended amount of sample for starting:

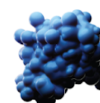
The DNA yield increases linearly with the amount of sample material used, 10-25mg of the sample is recommended as starting material.

Storage of Tissue samples

Fresh sample material or the samples that are immediately frozen and stored at -90°C to -20°C will yield 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 prolonged storage of tissue samples under these conditions.



Standard Operation Procedure

Genomic DNA Extraction From Tissue Using Zybio EXM-3000

Storage condition

DNA extraction kit can be kept at room temperature.

Kit Contents

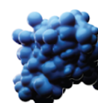
CONTENT	QUANTITY	STORAGE
Pre-Lysis buffer	3.5 mL	Room temperature
Lysis buffer	3.5 mL	Room temperature
Lysis-Binding Buffer (LBB)	10 mL	Room temperature
Proteinase K (lyophilized)	10 mg	-20°C (upon reconstitution)
Proteinase K Diluent (PKD)	500 mL	Room temperature
Wash Buffer I (WB1)	10 mL	Room temperature
Wash Buffer II (WB2)	6 mL	Room temperature
Wash Buffer III (WB3)	3.5 mL	Room temperature
Elution Buffer	3.5 mL	Room temperature

How to start

Items required by the user but not provided in the kit:

Equipment:

1. Zybio EXM-3000
2. Heat block
3. Microcentrifuge tube



Preparation of working solutions

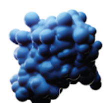
1. Reconstitute 10 mg of lyophilized Proteinase K powder by adding 0.5 mL of Proteinase K diluent. After reconstitution the Proteinase K solution is stored at -20°C.

DNA extraction from tissue - Plate description

BUFFER	VOLUME OF BUFFER PER WELL	WELL POSITION ON "U BOTTOM" DEEP WELL PLATE
Lysis Binding Buffer Proteinase K Solution Pre-treated tissue sample	500µL 25 µL 200 µL	Column 1 and 7
CamOxyl beads	200 µL	Column 2 and 8
Wash Buffer I	500 µL	Column 3 and 9
Wash Buffer II	300 µL	Column 4 and 10
Elution buffer	100 µL	Column 6 and 12

Before starting the preparation

- Pre-lysis Buffer may precipitate during storage. If necessary, warm the buffer container at 70 °C until the precipitate fully dissolves.
- Set an incubator or water bath to 56°C.



Protocol:

1. Sample preparation

Aliquot 25 mg of animal or human tissue into small pieces. Place the samples into micro-centrifuge tubes and proceed with step 2.

- *Grinding the tissues using a micro pestle into small pieces is recommended for rapid/efficient lysis of the tissue.*
- *Sample lysis time may be reduced by disrupting the sample using liquid nitrogen, a bead mill, or treated with a mechanical homogenizer.*
- *Ensure correct amount of starting material is used.*

2. Initial-lysis

Add 200 µL pre-lysis 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.

Transfer the tissue lysate to wells of column 1 and column 7 (containing the lysis binding buffer).

s(Note: Pipette the tissue sample gently from the microcentrifuge tube to the wells of column 1 and column 7 (containing the lysis binding buffer) gently, ensure proper transfer of the lysate into the deep-well plate).

***Samples may be incubated overnight. If the samples are not lysed completely, centrifuge the sample at 11000x g for 5 minutes and transfer the supernatant lysate.*

3. Place the filled Zybio plates on-to the Zybio platform and fit the magnetic-sleeves properly. Select protocol “Zybio-FFPE-B-200” and run the protocol.

4. After the protocol run, collect the eluated DNA in a DNAse, RNase- free microcentrifuge tubes and store the eluted DNA in -20°C.