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:

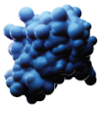
The DNA yield increases linearly with the amount of sample material used, 10-25mg of the sample is recommended as starting material.

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

Manual Genomic DNA extraction from Tissue using CamBeads

Kit contents

CONTENTS	QUANTITY (10 REACTIONS)	STORAGE
TL Buffer (TL)	2.2 mL	Room temperature
BL Buffer (BL)	2.2 mL	Room temperature
Proteinase K (lyophilized)	6 mg	-20°C (upon reconstitution)
Proteinase K Diluent (PLD)	0.5 mL	Room temperature
LB Buffer (LBB)	5.5 mL	Room temperature
CamBeads (CB)	0.2 mL	Room temperature
Wash Buffer I (WB1)	4.42 mL	Room temperature
Wash Buffer II (WB2)	3 mL	Room temperature
Elution Buffer (EB)	1.2 mL	Room temperature

How to start

Items required by the user:

- MagStand

Additional buffers to be prepared by the user:

- RNase A (10 mg/mL)
- 80% Ethanol

Preparation of working solutions

1. **Proteinase K solution** Reconstitute the lyophilized **Proteinase K** powder by adding 0.3 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.

Before starting the preparation

- Buffer TL may precipitate during storage. If necessary, warm the buffer container at 70 °C until the precipitate fully dissolves.
- Set a dry bath or water bath to 56°C.

Protocol for DNA extraction from tissue

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 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 the correct amount of starting material is used.*
2. Add **200 µl Buffer TL** and **25 µl proteinase K** to the microcentrifuge tube containing the pellet sample solution. Vortex vigorously and make sure the pellet is completely submerged and homogenized.
3. Incubate the microcentrifuge tube at **56°C for at least 30 min** or until the sample is completely lysed. Vortexing for **30 seconds every 20 minutes** will help to speed up the lysis process.

    - *Samples may be incubated overnight.*
    - *Additional Buffer TL 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 A (10 mg/mL) and incubated at RT for 10 min.*
4. Once the lysis is complete, centrifuge the samples at 11000x g for 5 minutes and proceed with the **supernatant**. Add **200 µL Buffer BL** to the supernatant and vortex the mixture vigorously (1 min).

    Note: Vigorous mixing is essential to obtain a high yield and purity of DNA.

5. Add **500 µL of LB Buffer** to the lysate and **20 µL of CamBeads** to the microcentrifuge tube. Vortex the tubes for 30 seconds between every 2-minute and 30 seconds intervals for a total time period of 10 minutes.
6. Place the tubes on a magstand. Wait for 1-2 minutes for the beads to settle on the wall of the magnet. Discard the lysate.

7. Add **600 µL of Wash Buffer I** to the beads and remove the tubes from the stand. Vortex the tube for 15 seconds to wash the beads and place the microcentrifuge tube back on the stand.
8. Once the beads have settled against the wall of the magnet, discard the supernatant.
9. Add **600 µL of Wash Buffer II** to the beads and remove the tubes from the stand. Vortex the tube for 15 seconds to wash the beads and place the microcentrifuge tube back on the stand.
10. Once the beads have settled against the wall of the magnet, discard the supernatant.
11. Add **600 µL of 80% Ethanol** to the beads and remove the tubes from the stand. Vortex the tube for 15 seconds to wash the beads and place the microcentrifuge tube back on the stand.
12. Once the beads have settled against the wall of the magnet, discard the 80% Ethanol solution. Air dry the beads at room temperature for 10 minutes.
13. Remove the tube from the magstand. When all traces of ethanol are removed and the beads are dry, add 100 µL of elution buffer to the tube.
14. Vortex the tube for 4- 5 minutes for efficient elution and transfer the tube to a magstand. Once the beads have settled against the wall of the magnet, collect the eluted DNA in a fresh DNase- RNase- free microcentrifuge tube and store it at -20°C