Nama Gene Azma

Tissue DNA Extraction Protocol

Important Notes:

- Wear gloves and lab coat when handling the buffers provided in this kit. They contain irritants.
- Do not add acidic solution or bleach directly to the sample preparation waste.
- The precipitates that appear during storage, especially at low temperature, in some buffers are easily dissolved by incubating the bottles at 60°C.
- Store Proteinase K at -20°C.
- Add 20ml absolute ethanol to Wash Buffer I and 40ml to Wash Buffer II.

* Set thermoblock or water bath at 55°C.
* Make sure that ethanol has been added into Wash Buffers.

1- Cut tissue into small pieces for rapid lysis and high yields.
2- Place the tissue (up to 15mg) in a 1.5ml microtube.
3- Add 200µl **Lysis Buffer** and 10µl **Proteinase K** respectively and mix immediately by vortexing.
4- Incubate samples at 55°C in a thermoblock until tissue pieces have completely lysed. (3hours-overnight)
Optional: Add 3µl RNase A (not provided in this kit) to the lysate, vortex and incubate at 55°C for 10 min.

* Set thermoblock or water bath at 70°C.

5- Add 200µl **Binding Buffer** & 10µl **Proteinase K** to the sample. Mix thoroughly by vortexing, and incubate at 70°C for 10 min.
6- Add 200µl of absolute ethanol to the sample and mix thoroughly by vortexing. Spin down briefly to remove any drops from inside of the lid.
7- Transfer the mixture into the spin column placed in a collection tube. Centrifuge for 1 min at 10000 rpm. Discard flow-through.
8- Place the column into a new collection tube, add 500µl **Wash Buffer I**, centrifuge for 1 min at 10000 rpm. Discard flow-through.
9- Place the column in a new 2ml collection tube, add 500µl **Wash Buffer II**, centrifuge for 1 min at 10000 rpm. Then proceed centrifugation at full speed (14000 rpm) for 2-3 min to dry the membrane completely.
10- Place the column in a clean 1.5ml microtube. Carefully open the lid of the column and apply 50-100µl preheated **Elution Buffer** to the center of the membrane.
11- Close the lid and incubate at 70° C for 2-5 min. Centrifuge at full speed (>12000 rpm) for 1 min.
12- Store eluted DNA at -20°C.