Standard Operation Procedure

Genomic DNA extraction from Whole Blood using CamBeads manually

Kit contents

CONTENTS	QUANTITY (10 REACTIONS)	STORAGE
BL Buffer (BL)	2.5 mL	Room temperature
Proteinase K (lyophilized)	6 mg	-20°C (upon reconstitution)
Proteinase K Diluent (PKD)	0.5 mL	Room temperature
Lysis-Binding Buffer (LBB)	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)	1.5 mL	Room temperature

How to start

Items required by the user:

- MagStand

Additional buffers to be prepared by the user:

- 80% Ethanol

Recommended Sample volume for starting

A starting sample volume of 200 µL of whole blood is suggested for blood DNA extraction. Whole blood samples collected in K2-EDTA and K3-EDTA vacutainers , stored at 4°C, frozen and at RT can be used for whole blood genomic DNA extraction.

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 3.6 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.

Protocol for genomic DNA extraction from whole blood

1. Add **30 µL of Proteinase K** solution in a microcentrifuge tube. Add **200 µL of whole blood** to it. To the whole blood add **200 µL of Buffer BL** and vortex it for 30- 40 seconds.
2. Incubate the sample at 70°C for 10 minutes in a water bath or a thermomixer.
3. Add **500 µL of LB Buffer** to the lysate and **15 µL of CamBeads** to the microcentrifuge tube.
4. Vortex the tubes for 7 minutes for effective DNA binding to the bead.

   NOTE: If continuous vortexing is not feasible, vortex for the tubes for 30 seconds between every 2 minutes and 30 seconds for a time duration of 7 minutes.
5. Place the tubes on a mag stand. Wait for 1-2 minutes for the beads to settle on the wall of the tube. Discard the lysate.
6. 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.
7. Once the beads have settled against the wall of the tube, discard the supernatant.
8. 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.
9. Once the beads have settled against the wall of the tube, discard the supernatant.
10. 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.
11. Once the beads have settled against the wall of the tube, discard the 80% ethanol solution. Dry the beads at 37°C for 2-3 minutes.
12. When all traces of ethanol are removed and the beads are dried thoroughly, add 100 µL of pre-heated **(at 70°C) elution buffer** to the tube.
13. Vortex the tube for 4- 5 minutes for efficient elution and transfer the tube back to the mag stand. Once the beads have settled against the wall of the tube, collect the eluted DNA in a fresh DNAse- RNAse free microcentrifuge tube and store it at -20°C.