DNeasy Blood & Tissue Handbook

Animal Blood

1. For blood with nonnucleated erythrocytes, follow step 1a; for blood with nucleated erythrocytes, follow step 1b; for cultured cells follow 1c.

1a. Nonnucleated: Pipette 20 ul proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 50-100 ul anticoagulated blood. Adjust the volume to 220 ul with PBS. Continue to step 2

**1b. Nucleated: Wash and resuspend PBMCs in 100uL PBS, add 20 ul proteinase K. Adjust the volume to 220 ul with PBS. Continue with step 2**.

1c. Culture cells: Centrifuge the appropriate number of cells (maximum 5X106) for 5 min at 300 x g. Resuspend the pellet in 200 ul PBS. Add 20 ul proteinase K. Continue with step 2.

1. Add 200 ul Buffer AL (without added ethanol). Mix thoroughly by vortexing and incubate at 56oC for 10 min.
2. Add 200 ul ethanol (96-100%) to the sample, and mix thoroughly by vortexing.
3. Pipette the mixture from step 3 into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at 8,000 rpm for 1 min. Discard the flow-through.
4. Add 500 ul of Buffer AW1, and centrifuge for 1 min at 8,000 rpm. Discard the flow-through.
5. Add 500 ul of Buffer AW2, and centrifuge for 3 min at 14,000 rpm to dry the DNeasy membrane. Discard the flow-through and the collection tube.
6. Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube, and pipette 100-200 ul Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 5 min, and then centrifuge for 1 min at 8,000 rpm to elute.

Paraffin-Embedded Tissue

1. Place a small section of paraffin-embedded tissue in a 2 ml microcentrifuge tube.
2. Add 1200 ul xylene. Vortex vigorously.
3. Centrifuge at full speed for 5 min at room temperature (15-25oC)
4. Remove supernatant by pipetting. Do not remove any of the pellet.
5. Add 1200 ul of ethanol (96-100%) to the pellet to remove residual xylene, and mix gently by vortexing.
6. Centrifuge at full speed for 5 min at room temperature.
7. Carefully remove the ethanol by pipetting. Do not remove any of the pellet.
8. Repeat steps 5-7 once.
9. Incubate the open microcentrifuge tube at 37oC for 10-15 min until the ethanol has evaporated.
10. Resuspend the tissue pellet in 180 ul Buffer ATL.
11. Add 20 ul proteinase K. Mix thoroughly by vortexing, and incubate at 56oC until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.
    1. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1-3 hours.
12. Vortex for 15 s. Add 200 ul Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 ul ethanol (96-100%), and mix again thoroughly by vortexing.
13. Pipette the mixture from step 12 (including and precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at 8000 rpm for 1 min. Discard flow-through.
14. Add 500 ul Buffer AW1, and centrifuge for 1 min at 8000 rpm. Discard flow-through.
15. Add 500 ul Buffer AW2, and centrifuge for 3 min at 14,000 rpm to dry the DNeasy membrane. Discard flow-through and collection tube.
16. Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube, and pipette 200 ul Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 8000 rpm to elute.