Extract DNA from Buffy Coat (Sigma Kit)

Preparation:

1. Defrost sample in 4℃ refrigerator for one day.

2. Dilute **Prewash Solution Concentrate** with 95-100% ethanol.

10 prep package	5.5 mL
70 prep package	27.5 mL
350 prep package	110 mL

3. Dilute **Wash Solution Concentrate** with 95-100% ethanol.

10 prep package	10 mL
70 prep package	80 mL
350 prep package	360 mL

4. Dissolve the **Proteinase K** with water

10 prep package	0.25 mL
70 prep package	0.5 mL
350 prep package	5.0 mL

Note: Do not combine the Proteinase K and Lysis Solutions for storage.

Procedure:

1. Turn on dry bath incubator first.
2. Label sample ID according to data sheet.

Note: From this step,

(1) all the wastes are biohazard

(2) change tips when treating different samples

3. Place **20 uL of Proteinase K** into a 1.5 mL microcentrifuge tube.
4. Add **50 uL Resuspension solution.**
5. Use wide bore pipette tip to take **150 uL of whole blood sample** to the tube.
6. Add **20 uL of Rnase A** and incubate for 2 mins at room temperature.
7. Add **200 uL of Lysis Solution C** to the sample and vortex thoroughly for 15 sec
8. Incubate at 55℃ for 10 min
9. Add **500 uL of Column Preparation Solution** to each column and centrifuge at 12,000 X *g* for 1 min. Discard the flow-through liquid.
10. Add **200 uL of ethanol** (95-100%) to the lysate from step 8.
    Mix thoroughly by vortexing for 5-10 sec.
11. Transfer the entire contents of the tube into the treated column and centrifuge at max speed for 1 min. Discard the collection tube containing the flow-through liquid and place the column in a new collection tube.
12. Add **500 uL of Prewash Solution** to the column and centrifuge for 1 min at max speed. Discard the flow-through liquid and collection tubes.
13. Add **500 uL Wash Solution** to the column and centrifuge for 3 min at max speed to dry the column. Discard the flow-through liquid, BUT keep the collection tube.
14. Use the same tube to centrifuge for 1 min at max speed. Discard the collection tube and flow-through liquid.
15. Add **200 uL Elution Solution** into the center of the column. Incubate for 5 min at room temperature.
16. Centrifuge for 1 min at max spped to elute the DNA.
17. Repeat step 15 and 16.
18. Measure DNA concentration.