Extract DNA from Buffy Coat (Sigma Kit)

**Preparation:**

1. Defrost sample in 4°C refrigerator for one day.

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

<table>
<thead>
<tr>
<th>10 prep package</th>
<th>70 prep package</th>
<th>350 prep package</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5 mL</td>
<td>27.5 mL</td>
<td>110 mL</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>10 prep package</th>
<th>70 prep package</th>
<th>350 prep package</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mL</td>
<td>80 mL</td>
<td>360 mL</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>10 prep package</th>
<th>70 prep package</th>
<th>350 prep package</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 mL</td>
<td>0.5 mL</td>
<td>5.0 mL</td>
</tr>
</tbody>
</table>

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°C 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 speed to elute the DNA.
17. Repeat step 15 and 16.
18. Measure DNA concentration.