# *AccuPrep*® Stool DNA Extraction Kit (K-3036)

## Ⅰ Before You Begin

1) Completely dissolve one vial of **Proteinase K** in **1,250 µl** of nuclease free water. For short term storage, dissolved Proteinase K should be stored at **4°C**. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at **-20°C**.
2) Add correct amount of **absolute ethanol** to **WA1 Buffer**.
3) Before starting extraction process, heat the **EA Buffer** at **56~60°C**.

## Ⅱ Experimental Protocol

1) Add **20 µl** of **Proteinase K** to a 1.5 ml or 2 ml tube.
2) Apply about **100~200 mg** of **stool sample** to the tube containing proteinase K.
3) Add **400 µl** of **SL Buffer** to the sample and mix immediately by vortex mixer. You must completely resuspend the sample to achieve maximum lysis efficiency.
4) Incubate at **60°C** for **10 min**.
5) After 10 min, centrifuge the tube at **13,000 rpm** for **5 min**, then transfer the supernatant to a new tube.
6) Add **400 µl** of **SB Buffer** and mix immediately by vortex mixer.
7) Incubate at **60°C** for **10 min**.
8) After 10 min, Add **100 µl** of **Isopropanol**, lightly vortex for about 5 sec.
9) Transfer the liquid into the Binding column tube (fit in a collection tube) not getting the lid wet.
10) Close the tube and centrifuge at **8,000 rpm** for **1 min**.
    **(Option)** If the liquid has not completely passed the column following centrifugation, then centrifuge again until the liquid completely passes through.
11) Discard the solution from the collection tube and reuse the collection tube.
12) Add **500 µl** of **WA1 Buffer** to the column, close the lid, and centrifuge at **8,000 rpm** for **1 min**.
13) Discard the solution from the collection tube and reuse the collection tube.
14) Add **500 µl** of **W2 Buffer**, to the column, close the lid, and centrifuge at **8,000 rpm** for **1 min**.
15) Discard the solution from the collection tube and reuse the collection tube.
16) Centrifuge once more at **13,000 rpm** for **1 min** to remove ethanol completely.
    **(Caution)** Make sure that there is no droplet hanging from the bottom of the Binding column. Residual W2 Buffer left in the Binding column may cause problems in later applications.

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17) Transfer the Binding column to a 1.5 ml tube for elution, add **50~200 µl** of **EA Buffer**, and let stand for 1 min to allow the buffer to permeate the column.
**(Option)** We recommend letting stand for about 5 min to increase DNA yield. You can also increase yield by heating the EA Buffer at about 60℃ before adding to the column.
18) Elute by centrifuge at **8,000 rpm** for **1 min**. The eluted DNA solution can directly be used, or stored at 4℃ for longer storage.

※ For more information, please visit www.bioneer.com and refer to the User's Guide of this kit.