7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
Email: info@abpbio.com

# EasySC Food DNA Purification Kit

## Catalog Number: D133-1, D133-2

**Table 1. Kit Components and Storage**

| Kit Component | D133-1 (50 preps) | D133-2 (200 preps) | Storage | Stability |
|---|---|---|---|---|
| **Buffer AP1** | 25 mL | 100 mL | RT | The product is stable for one year when stored as directed. |
| **Buffer AP2** | 10 mL | 40 mL | RT | |
| **Buffer AP3*** | 15 mL | 2×30 mL | RT | |
| **Buffer AW2*** | 15 mL | 2×25 mL | RT | |
| **Buffer AE** | 15 mL | 30 mL | RT | |
| **Proteinase K (20 mg/mL)** | 1 mL | 4×1 mL | -20 °C | |
| **Mini Column** | 50 | 200 | RT | |
| **2 mL Collection Tube** | 50 | 200 | RT | |

***** Prior to use, add absolute ethanol to **Buffer AP3, Buffer AW2** according to the bottle label.

## Product Description

EasySC Food DNA Purification Kit is designed for the rapid and reliable isolation of high-quality genomic DNA from raw or processed food from plant, animal, or mixed origins. Up to 100 mg of raw or processed food can be processed in less than 60 minutes. This kit uses a proprietary buffer system to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from food sample. There are no organic extractions, and time-consuming precipitation steps. Purified DNA is suitable for PCR, restriction enzyme digestion, and hybridization techniques.

## Features

- ❖ Fast – DNA purification process in less than 60 min.
- ❖ Safe – No organic extraction, no ethanol precipitation.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

## Things to do before starting

- Buffer AP1 and Buffer AP3 may form precipitates upon storage. If necessary, warm to 50°C to redissolve.
- Buffer AP3 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 65°C.

## Purification Protocol

1. Food disruption.
    - Solid food: Grind the solid food to a fine powder under liquid nitrogen using a mortar and pestle. Transfer 100 mg of the food powder into a 2 mL microcentrifuge tube. Do not allow the sample to thaw.
    - Semi-solid food: Transfer the semi-solid food into a 2 mL microcentrifuge tube. Centrifuge at 13,000 x g for 5 min. Carefully remove the supernatant and discard. If the sample contains lot of impurities, wash with PBS buffer a few times.

      **Note:** The volume of the food sample used depends on the solid content of the food sample. The volume of the precipitate after centrifuge should not exceed 0.2 mL. For food samples without precipitation after centrifugation, add 0.7 volume of isopropanol and 0.1 volume of 3 M NaOAc to the sample, mix and centrifuge to collect the precipitate.
2. Immediately add 400 µL Buffer AP1 and 20 µL Proteinase K solution to the disrupted food sample and vortex vigorously.
3. Incubate the mixture for 1-3 hr at 65°C. Mix every 20 min during incubation by inverting tube.
4. Add 130 µL Buffer AP2 to the lysate. Mix and incubate for 10 min on ice.

   **Note:** This step precipitates detergent, proteins and polysaccharides.
5. Centrifuge the lysate at 14,000 x g for 5 min. Carefully transfer 400 µL the cleared lysate to a new 2 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet.
6. Add 600 µL Buffer AP3 to the cleared lysate, and mix by pipetting.

   **Note:** A precipitate may form after the addition of Buffer AP3, pipette up and down several times to disperse the sediment. Buffer AP3 must be diluted with absolute ethanol according to the bottle label before use.
7. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer half mixture including any precipitate that may have formed from step 6 into the DNA Mini column. Centrifuge at 8,000 x g for 1 min.
8. Discard the filtrate and reuse the collection tube. Transfer remaining sample from step 6 to the DNA Mini Column. Centrifuge at 8,000 x g for 1 min.
9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 8,000 x g for 1 min.

   **Note**: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
10. Repeat step 9 for a second wash step.
11. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    **Note**: This step is critical for removing of trace ethanol that may interfere with downstream applications.
12. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 10,000 x g for 1 min.

    **Note**: To improve the yield, repeat this step for a second elution step.
13. Discard the column and store the DNA at -20ºC.

## Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Column clogged | • Carryover of particulate material: Ensure that no particulate material is transferred when supernatants are transferred to new microcentrifuge tube prior to addition of Buffer AP3.<br>• Insufficient centrifugation: Increase the *g*-force and centrifugation time.<br>• Lysate too viscous: Reduce the amount of starting material and/or increase the amounts of Buffer AP1and Buffer AP2. |
| Low yield | • Insufficient disruption: Ensure that the starting material is completely disrupted.<br>• Insufficient lysis: Reduce the amount of starting material and/or increase the amounts of Buffer AP1 and Buffer AP2.<br>• Incorrect binding conditions: Make sure that the correct amount of Buffer AP3 is added to adjust the binding conditions correctly.<br>• DNA still bound to the membrane: Increase the volume of Buffer AE to 200 µL, and incubate the column at 50°C for 5 min before centrifugation.<br>• Column matrix lost binding capacity during storage: Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x g for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x g for 30 seconds. Discard the filtrate. |
| Poor performance in downstream applications | • Salt contamination: Repeat Buffer AW2 wash twice.<br>• Ethanol contamination: incubate the column at 56°C for 5 min to dry the membrane completely. |