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# EasyMag DNA Purification Kit from animal cells

## Catalog Number: D118-1, D118-2

**Table 1. Kit Components and Storage**

| Kit Component | D118-1 (50 preps) | D118-2 (200 preps) | Storage | Stability |
|---|---|---|---|---|
| **Buffer AL** | 15 mL | 60 mL | RT | The product is stable for one year when stored as directed. |
| **Buffer DW1*** | 15 mL | 53 mL | RT | |
| **Buffer DW2*** | 15 mL | 2×25 mL | RT | |
| **Buffer AE** | 15 mL | 30 mL | RT | |
| **MagBinding Beads** | 2.5 mL | 10 mL | 2-8 $^{o}$C | |

***** Prior to use, add absolute ethanol to **Buffer DW1, Buffer DW2** according to the bottle label.

## Product Description

EasyMag DNA Purification Kit from animal cells provides rapid total DNA isolation from cultured cells. The kit can be processed manually or on an automated platform. Phenol/chloroform extraction, and time-consuming steps such as precipitation with isopropanol or ethanol have been eliminated. The isolated DNA is ready for applications such as PCR, Southern blotting, or restriction enzyme digestion.

## Features

- ❖ Fast – DNA purification process in less than 30 min.
- ❖ Safe – No Phenol/chloroform extractions.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

## Things to do before starting

- Buffer AL may form precipitates upon storage. If necessary, warm to 50°C to redissolve.
- Buffer DW1 and Buffer DW2 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.
- Prepare RNase A solution (10 mg/mL).

## Purification Protocol

1. Prepare the cell suspension using one of the following methods:
    - Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation. Wash the cells with cold PBS (4°C). Resuspend cells in 250 µL PBS. Proceed to Step 2.
    - For cells grown in suspension, pellet 5 x $10^6$ by spinning at 1,200 x g in a centrifuge tube. Aspirate and discard the supernatant, and wash the cells once with cold PBS (4°C). Resuspend cells in 250 µL PBS. Proceed to Step 2.

- For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or by scraping with a rubber policeman. Wash cells twice with cold PBS (4°C). Resuspend the cells in 250 µL PBS. Proceed to Step 2.

2. Add 250 µL Buffer AL to the sample. Vortex at maximum speed for 15-20 seconds, and incubate at 65°C for 15-30 minutes. Vortex briefly once during incubation.

   **Note**: Buffer AL may be precipitated during storage, if happen, heat it at 50$^{o}$C to dissolve. If RNA need be removed, add 10 µL RNase A Solution (10 mg/mL) to the sample.

3. Add 250 µL isopropanol and 50 µL MagBinding Beads to the sample, and vortex for 30 second.
4. Incubate with shaking at RT for 5 min.
5. Transfer the tube to a magnetic rack for an additional 2 min or until beads pellet and supernatant is cleared. With the tube on the magnetic rack remove the supernatant and discard.
6. Add 600 µL of Buffer DW1 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

   **Note**: Buffer DW1 must be diluted with absolute ethanol according to the bottle label before use.

7. Add 600 µL of Buffer DW2 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

   **Note**: Buffer DW2 must be diluted with absolute ethanol according to the bottle label before use.

8. Repeat step 7 for a second wash step.
9. Leave the tube on the magnetic rack, open the lid and air dry the beads at RT for 10-15 min.

   **Note**: This step is critical for removing of trace ethanol that may interfere with downstream applications.

10. Add 100 µL Buffer AE to the tube, resuspend the beads by vortexing. Incubate at 55ºC for 5 min, vortex for 15 seconds every 1-2 min for 5 min.
11. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
12. Store the DNA at -20ºC.

## Troubleshooting

| Problem | Possible cause and suggestions |
|---|---|
| Low yield | • DNA still bound to beads: Increase the volume of Buffer AE to 200 µL, and incubate the bead suspension at 50°C for 5 min.<br>• Improper Washing: Buffer DW1, buffer DW2 must be diluted with absolute ethanol before use.<br>• Inefficient lysis: Mix the sample thoroughly with Buffer AL and increase incubation time. |
| Poor performance in downstream applications | • Salt contamination: Remove any residual liquid with a pipettor after each wash step.<br>• Ethanol contamination: Ensure the beads are completely dried before elution. |