Easy Plasmid DNA Miniprep Kit: EF

AM1004-2-50P, AM1004-2-100P, AM1004-2-250P

PRODUCT OVERVIEW

- **High-Capacity Plasmid Purification** — Efficiently isolates 4–40 µg of plasmid DNA from 1–10 ml overnight bacterial cultures, depending on plasmid copy number and growth conditions.
- **Rapid Spin-Column Workflow** — Uses a silica-membrane spin column with non-toxic buffers for fast plasmid DNA purification in under 30 minutes with minimal hands-on time.
- **Enhanced Contaminant Removal** — Includes unique binding chemistry with optimized wash steps that eliminate proteins, RNA, and endotoxin impurities, improving DNA quality for sensitive downstream applications.
- **Broad Downstream Compatibility** — Produces plasmid DNA suitable for eukaryotic transfection and performs reliably in other demanding uses such as *in vitro* expression, sequencing, cloning, and enzymatic reactions.

COMPONENTS

Components	AM1004-2-50P	AM1004-2-100P	AM1004-2-250P	Storage
Buffer PE1	14 ml	28 ml	70 ml	4° C
Buffer PE2	14 ml	28 ml	70 ml	RT
Buffer PE3	17 ml	34 ml	85 ml	RT
Buffer PE4	14 ml	28 ml	70 ml	RT
Buffer PE5	40 ml	80 ml	200 ml	RT
Buffer PE6 (concentrate)	8 ml	16 ml	40 ml	RT
Buffer PE7	5 ml	10 ml	20 ml	RT
RNase A Solution	70 µl	140 µl	350 µl	-20° C
Plasmid Columns with Collection Tubes	50	100	250	RT

STORAGE CONDITIONS

The Easy Plasmid Miniprep Kit: EF should be stored at room temperature (15°C to 25°C), except for RNase A, which should be stored at -20°C. If a -20°C freezer is not available, store the RNase A tube at 4 °C, and it is stable for up to 2 years. If you notice any precipitation in the buffers, it can be dissolved by gently warming them to 37-45 °C.

TO BE SUPPLIED BY THE USER

- Standard microbiological equipment for cultivating and collecting bacteria
- A centrifuge with a capacity to sustain a minimum of 12,000 rpm (~15,000 *x g*)
- 96-100% (v/v) ethanol
- Centrifuge tubes

PREPARATION OF WORKING BUFFERS

BUFFER PE1: Combine the entire supplied RNase A solution with Buffer PE1. After the addition of RNase A, Buffer PE1 remains stable for up to 18 months when stored at 2°C to 8°C.

BUFFER PE6: To prepare the working Column Wash Buffer PE6, add 31 ml of 96-100 % (v/v) ethanol. Make sure to label the bottle to indicate the addition of ethanol. After each use, securely seal the bottle cap to avoid evaporation.

PROCEDURE

1. Centrifuge up to 1-5 ml of overnight culture, typically derived from a single bacterial colony, for 3 minutes at >8000 rpm (~7000 *x g*). Discard the supernatant by pouring it off, then gently blot the inverted tube on a paper towel to eliminate any excess media.

2. Completely resuspend the pellet in **250 µl** of **Buffer PE1**. Make sure buffer PE1 contains RNase A.

3. Add **250 µl** of **Buffer PE2** to the sample and gently invert the tube 8-10 times to ensure thorough mixing. Incubate at room temperature for 3-4 minutes. Do not extend the incubation beyond 5 minutes or vortex during lysis.

4. Add **300 µl** of **Buffer PE3** and mix the tubes by inverting 8-10 times. Do not vortex.

5. Centrifuge for 10-15 min at >12,000 rpm (~15,000 *x g*) in a table-top microcentrifuge at 4° C. If a cooling centrifuge is not available, this step can also be performed at room temperature.

6. Transfer **exactly** 600 µl lysate to a fresh microcentrifuge tube. Add **250 µl** of **Buffer PE4** to the cleared lysate and mix the tubes gently by inverting 6-8 times. If the cleared lysate is less than 600 µl, reduce the volume of Buffer PE4 proportionally.

7. Place a Spin Column into the Collection Tube and transfer the entire cleared lysate into the Spin Column. Centrifuge at >6000 x *g* for 30–60 s.

8. Discard the flow-through and reinsert the Spin Column into the Collection Tube.

9. Add **700 µl** of **Buffer PE5** to the Spin Column and centrifuge at >6,000 x *g* for 30–60 s. Discard the flow-through and reinsert the column into the Collection Tube.

10. Add **700 µl** of **Buffer PE6** and centrifuge at >6,000 x *g* for 30–60 s. Make sure that Buffer PE6 is supplemented with ethanol. Discard the flow-through and reinsert the Spin Column into the Collection Tube.

11. Centrifuge at >6,000 x *g* for 1 minute to dry the Spin Column. Insert the Spin Column into a fresh and sterile 1.5 ml microcentrifuge tube.

12. Add **50-100 µl** of pre-warmed **Buffer PE7** or Nuclease-Free Water to the Spin Column. Let it sit for 1-2 min at room temperature, and Centrifuge at >6,000 x *g* for 2 minutes.

13. Discard the column and store the eluted DNA at –20°C or below.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Presence of RNA	RNase A was not added to Buffer Ps1	Before use, ensure RNase A is added to Buffer PE1
Plasmid bands smeared on agarose gel	Plasmid DNA degradation	Keep plasmid preps on ice or frozen to avoid degradation
Presence of genomic DNA	1. Overgrown bacterial cultures 2. Prolonged incubation (>5 min) after Buffer PE2 addition. 3. Vigorous agitation or vortexing after adding buffer PE2 or PE3.	1. Avoid overgrowing cultures 2. Limit incubation after Buffer PE2 to ≤5 min 3. Do not vortex or agitate vigorously after adding buffer PE2 or PE3.
Low yields of DNA	1. Ethanol (96–100%) not added to Buffer PE6. 2. Column overloaded 3. SDS in Buffer PE2 precipitated 4. Incorrect elution conditions 5. Plasmid lost in the host 6. Low copy plasmid	1. Add 96–100% ethanol to Buffer W2 before use 2. Reduce loading volume or culture density 3. If SDS precipitates, incubate Buffer M2 at 30–40 °C for 5 min and mix well 4. Add Buffer BE directly to the center of the column 5. Use a fresh bacterial culture 6. Use a bigger culture volume and increase buffer PE1 to PE4 accordingly.