Plasmid DNA Extraction Kit **Cat. No. 10665-0, 10665-1, and 10665-2**

INTRODUCTION

Our Plasmid DNA Extraction Kit isolates high quality plasmid DNA from 1-5 mL *E. coli* bacterial cultures. Our kit is supplied with an enhanced DNA binding columns that produces high yields of plasmid. This quick and easy method adapted in our kit eliminates toxic phenol/chloroform extractions or lengthy ethanol precipitations. The plasmid DNA extracted with our kit is ready to use for downstream applications, such as restriction enzyme digestion, sequencing, subcloning and *in vitro* transcription. The plasmid yields are typically up to 20µg/prep with our kit.

ITEMS SUPPPLIED IN THE KIT

Items	**Cat. No. 10665-0** (50 Preps)	**Cat. No. 10665-1** (100 Preps)	**Cat. No. 10665-2** (200 Preps)
Suspension Buffer	15 mL	30 mL	60 mL
Lysis Buffer	25 mL	50 mL	100 mL
Neutralizing Buffer	25 mL	50 mL	100 mL
DNA Wash Buffer*	20 mL*	2 x 20 mL*	4 x 20 mL*
Plasmid Mini Columns with Collection Tube	50	100	200
SP-RNase	0.5 mL	0.5 mL	0.5 mL
TE Buffer	10 mL	10 mL	10 mL

Storage: The kit is shipped at ambient temperature. Upon arrival, store SP-RNase at -20°C and all other items may be stored at 4^0C/RT as marked on the label. The kit is stable for 12 months if stored as recommended.

NOTE: *If a precipitate forms in the buffers, particularly Lysis Buffer due to inappropriate storage, warm the buffers to room temp to dissolve the precipitate.*

Additional Items Needed

1. High speed microcentrifuge
2. 1.5ml Centrifuge tubes
3. Molecular Grade Ethanol 95%

PREPARATION BEFORE USE

I. Add 50µl SP-RNase per 10ml Cell Suspension Buffer and after addition, store the Suspension Buffer at 4 ^{0}C. The Suspension Buffer with added SP-RNase is stable for up to 6 months, if stored as recommended.

II. *To each bottle of 20 mL DNA Wash Buffer, add 80 mL Molecular Grade Ethanol. Chill the DNA Wash Buffer to -20°C before use.

III. Chill the Neutralizing Buffer on ice prior to use, DO NOT STORE AT 4°C.

IV. Warm the TE Buffer to 55-60°C before use.

Plasmid DNA Extraction Kit Protocol

NOTE: *Determine the optimal growth conditions, media, and antibiotic, etc., for each bacterial strain and plasmid. The bacterial culture should be harvested while bacteria are rapidly expanding and not after populations plateau or decline, since bacterial overgrowth can hamper plasmid yield. In general, the OD* A_{600} *readings of 1.5-2.0 provides maximum yield of high-quality plasmid DNA.*

1. Take 2-5 mL bacterial cell culture grown overnight with OD_{600} >1.5 and centrifuge at 7,000x g for 5 minutes. Discard the supernatant carefully without disturbing the pellet.
2. Ensure that the RNase was added into the Suspension Buffer, before. Add 250µl of Suspension Buffer with RNase to the bacterial pellet and re-suspend.
3. Add 250µl Lysis Buffer and mix it gently by inverting the tube a few times until the lysate is clear, DO NOT VORTEX. To ensure complete RNA digestion, incubate the tube for 3-4 minutes at room temperature. Do not exceed incubation for more than 5 minutes and DO NOT VORTEX.
4. Add 350µl chilled Neutralizing Buffer and gently invert 8-10 times to mix it completely. The lysate will turn into a thick white precipitate.
5. Centrifuge for 15 minutes at 15,000x g.
6. Ensure the Plasmid Mini column is in a collection tube and apply the supernatant on to the column. Centrifuge for 30-60 seconds at maximum speed and discard the flow through.
7. Wash the column by adding 500µl DNA Wash Buffer and centrifuge for 30-60 seconds at maximum speed. Discard the flow through. Repeat this step once.
8. Perform a final spin of the column for 60 seconds to remove any residual DNA Wash from the sides of the column.
   NOTE*: Place the columns at 37-55°C for 10-30 minutes to ensure all residual alcohol is removed. This helps eliminate issues with the residual alcohol, including samples leaving agarose gel wells during loading.*
9. Place the column in a clean 1.5ml tube and elute the plasmid DNA from the column by adding 50µl pre-warmed TE Buffer directly to the column. Incubate for 2 minutes at room temperature only then centrifuge for about 60 seconds. The eluted plasmid is ready for restriction digestion, sequencing, and other downstream applications.

RELATED PRODUCTS

1. **Genomic DNA Extraction Kit – Universal, 50 and 100 Preps (Cat. No. 10640 and 10640-1)**
   For extracting genomic DNA from animal tissues, cell culture, blood, Rodent tail, hair, and gram (-) bacteria.
2. **Genomic DNA Extraction Kit– Gram (+) Bacteria/Yeast/Fungi - 50 Columns (Cat. No. 10645)**
   Provides a fast and easy way to purify DNA from various Gram-positive bacteria, yeast, fungal tissue and fungi.
3. **Protein Extraction Buffers/ Kits (Cat. No. 10450, 10451, 10452, 10455 and 10457)**
   For extracting proteins from Bacteria, Insects & Mammalian cells, and Tissues
4. **Protease Inhibitor Cocktails (Cat. No. 10471, 10472, 10473, 10474 and 10475)**
   For inhibiting protease activity in the protein extracts: General, Bacterial, Mammalian, Plant and Recombinant.
5. **Phosphatase Inhibitor Cocktails (Cat. No. 10507, 10508, 10509)**
   For inhibiting phosphatases: serine/threonine (Ser/Thr), protein tyrosine, acid and alkaline phosphatase activities in the protein extracts.
6. **BCA Protein Assay Kit (Cat. No. 10477 & 10477-1)**
   For protein estimation.
7. **SDS-PAGE Sample Loading Buffer (Cat. No. 10502 & 10502-1**)
   For loading protein samples onto the gel
8. **SDS-PAGE Running Buffer (Cat. No. 10501 & 10501-1)**
   For running protein samples on SDS-PAGE