TIANGEN®

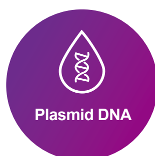
TGuide Smart Mini Plasmid Kit

(Prefilled 96-Deepwell plate)

TECHNICAL MANUAL

Cat. no. GDP641-E

Note: To use the TGuide Smart Mini Plasmid Kit, you must have the TGuide Smart Mini Plasmid (program no. DP641) installed on the TGuide S16/S32 pro Nucleic Acid Extractor.



This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetic

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Appendix 5

TGuide Smart Mini Plasmid Kit
(Prefilled 96-Deepwell plate)

Cat. no. GDP641-E

Kit Contents

Contents	GDP641-E (96 preps)
Buffer P1	60 ml
Buffer P2	60 ml
Buffer P4	60 ml
Plasmid DNA Reagents	6 plates
RNase A (10 mg/ml)	600 μl
TGuide Smart Tip Comb	12 Pcs

Plasmid DNA extraction reagents composition

Column 1/7	Column 2/8	Column 3/9	Column 4/10	Column 5/11	Column 6/12
Buffer IC	Buffer RDP	Buffer PWP	Buffer PWP	Buffer TB	MagAttract Suspension GSP1
300 μl	600 μl	800 μl	600 μl	100 μl	400 μl

Storage condition

The kit can be stored for 12 months at room temperature (15~30°C) under dry conditions. If the solution produces a precipitate, it should be placed at 37°C to dissolve the precipitate before use. Solution P1 should be stored at 2~8℃ after adding RNase A. It can be stored stably for 6 months. Individually packaged RNase A can be stored at room temperature (15~30℃) for 12 months.

Product

This kit is designed to isolate and purify 2~30 μg of high-quality plasmid DNA from 1~5 ml of bacterial solution using magnetic beads with unique isolation effects and a unique buffer system. The uniquely embedded magnetic beads have a strong affinity for plasmid DNA under certain conditions. When the conditions change, the magnetic beads release the adsorbed plasmid DNA, which can achieve the rapid isolation and purification of plasmid DNA. They can maximize the removal of proteins and other impurities, thus ensuring the purity of the extracted plasmid. Plasmid DNA extracted with this kit can be used in a variety of molecular biology experiments, such as enzymatic cleavage, sequencing, library screening,ligation and transformation.

Features

- **Simple and fast:** Ultra-pure plasmid DNA can be obtained within 1h.
- **Widely applicable:** Suitable for both high copy plasmids and low copy plasmids.
- **Safe and non-toxic:** No toxic organic reagents such as phenol/chloroform are required.
- **High purity:** The plasmid DNA obtained is of high purity and can be directly used in downstream experiments such as enzyme digestion and sequencing.

Notes

1. Before using Buffer P1, add RNaseA (Buffer P1:RNase A = 100:1), mix well, and store at 2~8℃.
2. Before use, Buffer P2 and P4 can be placed in a 37℃ water bath for a few minutes if appear cloudy. Solutions P2 and P4 should be tightly capped immediately after use.
3. All centrifugation steps were performed at room temperature.
4. The amount of plasmid extracted is depending on the culture concentration of bacteria, host bacteria, and plasmid copy number. If extract low-copy plasmids, customer can increase the volume of bacteria culture to 10 ml.

Operational steps

1. Prefilled 96-Deepwell plate

1.1 Take out a Prefilled 96-Deepwell plate and invert it to re-suspend the magnetic beads; Gently shake to concentrate the reagent and magnetic beads to the bottom of the plate. Before use, remove sealing film carefully to avoid liquid spatter or spills.

1.2 Add proper volume (60~100 μl) of elution buffer TB to column 5/11 of the plate.

2. Pre-treatment of bacterial samples

2.1 Take 1~5 ml of the overnight culture, add it to a centrifuge tube, centrifuge at 12,000 rpm (~13,400×g) for 2 min, and aspirate the supernatant as much as possible. (When there are more bacterial fluids, you can collect the bacterial pellets into a centrifuge tube by centrifugation several times).

2.2 Add 250 μl Buffer P1 to the centrifuge tube with the bacterial pellet **(check that RNase A has been added)**, and use a pipette or vortex to thoroughly suspend it.

Note: The presence of bacterial clumps that are not thoroughly mixed will affect lysis and result in low extraction volume and purity.

2.3 Add 250 μl Buffer P2 to the centrifuge tube and gently turn it up and down 6~8 times to fully lyse the bacteria.

Note: Mix gently, do not shake vigorously, so as not to interrupt the genomic DNA, resulting in genomic DNA fragments mixed in the extracted plasmid. At this point, the bacterial solution should become clear and viscous, and the time used should not exceed 5 min to avoid damage to the plasmid. If it does not become clear, the lysis may be incomplete due to too many organisms and the amount of organisms should be reduced.

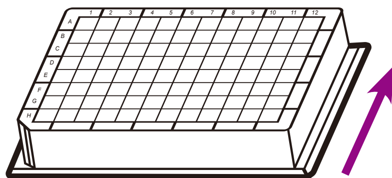
2.4 Add 250 μl Buffer P4 to the centrifuge tube and immediately mix thoroughly by gently turning up and down 6~8 times, at which point a white flocculent precipitate will appear. Centrifuge at 12,000 rpm (~13,400×g) for 5 min and proceed to 3.1.

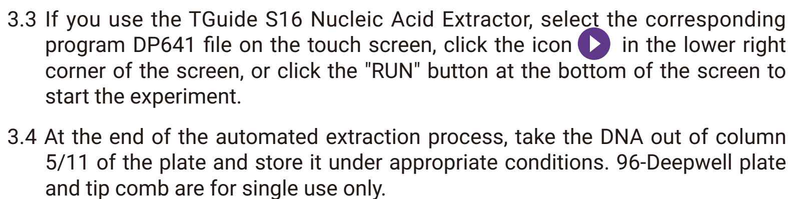
Note: Buffer P4 should be mixed immediately after addition to avoid localized precipitation. If there is still a tiny white precipitate in the supernatant, the supernatant can be taken after centrifugation again.

3. Operation steps of TGuide S16 Nucleic Acid Extractor

3.1 Add 600 μl above supernatant to column 1/7 of the plate.

3.2 Place the reagent plate on the base in the TGuide S16 Nucleic Acid Extractor. Insert the Tip Combs into the slots to ensure that they are well connected and firmed.





3.3 If you use the TGuide S16 Nucleic Acid Extractor, select the corresponding program DP641 file on the touch screen, click the icon ▶ in the lower right corner of the screen, or click the "RUN" button at the bottom of the screen to start the experiment.

3.4 At the end of the automated extraction process, take the DNA out of column 5/11 of the plate and store it under appropriate conditions. 96-Deepwell plate and tip comb are for single use only.

Appendix

The automated plasmid DNA extraction procedure is shown in the following table

Table 1

Step	Hole site	Step name	Mix time (min)	Mix speed	Dry time (min)	Volu-me (μl)	Temp (°C)	Seg-ments	Everytime (sec)	Magne-tization time (sec)	Cycle	Magnet speed (mm/s)
1	1	Lysis	2	8	0	900	--	1	0	0	0	--
2	6	Collect beads	0	--	0	400	--	5	3	0	2	2.5
3	1	Bind	3	8	0	900	--	5	3	0	2	2.5
4	2	Wash 1	2	7	0	600	--	5	3	0	2	2.5
5	3	Wash 2	2	7	0	800	--	5	3	0	2	2.5
6	4	Wash 3	2	7	6	600	--	5	3	0	2	2.5
7	5	Elution	5	8	0	100	56	5	5	0	2	2.5
8	6	Discard	0.5	5	0	400	--	1	0	0	0	--

Plasmid DNA concentration and purity test

The plasmid DNA obtained can be tested for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. When the concentration and purity of extracted DNA is measured by UV spectrophotometer, there is a significant absorption peak at OD_{260} (OD_{260} value of 1 corresponds to about 50 μg/ml double-stranded DNA, 40 μg/ml single-stranded DNA) and the OD_{260}/OD_{280} ratio should be 1.7~1.9.