TIANGEN®

TGuide Smart Endofree Mini+ Plasmid Kit 1 (5-20 ml)

(Prefilled single sample cartridge)

TECHNICAL MANUAL

Cat. no. DP641-D-E1

Note: To use the TGuide Smart Endofree Mini+ Plasmid Kit 1, you must have the TGuide Smart Endo-free Mini Plus 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

Table Contents

TGuide Smart Endofree Mini+ Plasmid Kit 1

(5-20 ml)

Cat. no. DP641-D-E1

Kit Contents

Contents	DP641-D-E1 (48 preps)
RNase A (100 mg/ml)	300 μl
Buffer P1	30 ml
Buffer DP2	15 ml
Buffer DP4	15 ml
Endotoxin Removal Buffer ER	4 ml
Plasmid DNA Reagents	48 Pcs
TGuide Smart Tip Comb	12 Pcs

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, which can be stable for 6 month. Individually packaged RNase A can be stored at room temperature for 12 months.

Product

This kit uses magnetic beads with unique separation properties and a unique buffer system to isolate and purify 2~100 μg of high quality plasmid DNA from 5~20 ml of bacterial broth. The uniquely embedded magnetic beads have a strong affinity for plasmid DNA under certain conditions, and when the conditions are changed, the magnetic beads release the adsorbed plasmid DNA, which can achieve the purpose of rapid isolation and purification of plasmid DNA, and 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 45 min.
- **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. Add RNase A to solution P1 before use (add all RNase A provided in the kit), mix well, and store at 2~8°C.
2. Before use Buffer DP2 and DP4 can be placed in a 37℃ water bath for a few minutes if appear cloudy. Solutions DP2 and DP4 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.

Operational steps

1. Prefilled single sample cartridge

1.1 Take out a prefilled single sample cartridge and invert it to re-suspend the magnetic beads; Gently shake to concentrate the reagent and magnetic beads to the bottom of the cartridge. 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 the 5th well of the cartridge.

2. Pre-treatment of bacterial samples

2.1 Take 5~20 ml of the overnight culture and add it to a centrifuge tube, centrifuge at 8000 rpm for 5 min and aspirate the supernatant as much as possible (when there is a large amount of bacteria, you can collect the bacterial pellet into a single centrifuge tube by centrifuging several times).

2.2 Add 500 μl Buffer P1 (check that RNase A has been added) to the centrifuge tube with the bacterial pellet, and thoroughly suspend it using a pipette or a vortex.

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 solution DP2 into the centrifuge tube, and gently turn it up and down for 6~8 times to make the bacterium fully lysed.

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 taken should not exceed 5 min in order 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 DP4 into the centrifuge tube, immediately turn up and down gently 6-8 times, mix well, then a white flocculent precipitate will appear.Centrifuge at 12,000 rpm (~13,400×g) for 5 min and proceed to 3.1.

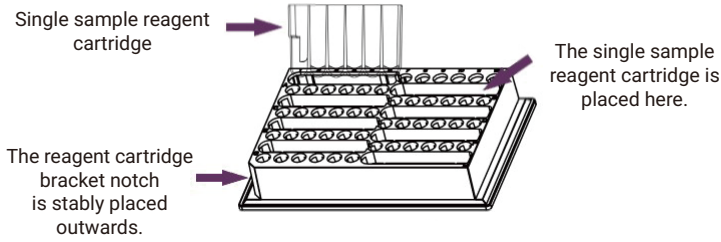
Note: DP4 should be mixed immediately after addition to avoid localized precipitation. If there is still a tiny white precipitate in the supernatant, the supernatant can be taken after centrifugation again.

3. Operation steps of TGuide S16 Nucleic Acid Extractor

3.1 Add 600 μl above supernatant and 60 μl Endotoxin Removal Solution ER to the 1st well of the cartridge and place cartridges on the reagent tank bracket of TGuide S16 Nucleic Acid Extractor.

3.2 Place the reagent tank bracket on the plate base in the TGuide S16 Nucleic

Acid Extractor. Insert the Tip Combs into the slot of the Tip Comb 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 the 5th well of the cartridge and store it under appropriate conditions. Single sample reagent cartridge 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)	Volume (μl)	Temp (°C)	Segments	Everytime (sec)	Magnetization 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.