cccDNA Purification Kit

Catalog Number：220501-100 and 220502-100

Product Description

Covalently closed circular DNA (cccDNA) is the natural form of most plasmids, it is also a key replicative intermediate in the life cycles of some viruses like HBV. In higher organisms including human, cccDNA is called extrachromosomal circular DNA (eccDNA)，which play a role in eliciting innate immune responses, mediating cell-to-cell communication, advancing genetic heterogeneity, compensating for genetic loss, aging, regulating gene expression and molecular sponging. In addition, cell-free eccDNAs in circulating system could be used as novel biomarkers for the diagnosis and prognosis of cancer (1).

The isolation of cccDNA from non-cccDNA is crucial to study cccDNA. Various isolation methods have been reported. Several techniques exploit the structural differences between chromosomal and circular DNA and separate them by SDS lysis-salt precipitation (2) or high-speed ultracentrifugation in cesium-chloride gradients (3). Most methods encompass extraction of total DNA purification followed by several rounds of enzymatic elimination of non-cccDNA and a final step of cccDNA extractions. The enzyme used includes exonuclease III which digest linear and open circular forms of DNA (4) , Plasmid-Safe DNase (5 -7) which preferentially hydrolyzes double-stranded linear DNA (dslDNA) and, with a lower efficiency, linear and closed circular single-stranded DNAs (ssDNAs), a combination of exonuclease I and exonuclease III which degrade DNA strands with free 3′ ends (8),and T5 Exonuclease (9),which specifically digest nicked double-stranded circular DNA.

This product is designed to purify cccDNAs from total DNA samples or partially purified cccDNA samples. It has the following features:

1. One step of extraction，simple to perform.
2. Minimal non-cccDNA contamination.
3. Maximal cccDNA recovery rate.
4. No enzymatic digestion.
5. Applicable to cccDNA of any size.
6. Compatible with silica-based purification (in both column format and magnetic beads format).
7. Enough for 100 times of purifications.
8. Research use only.

Input Sample Requirements

- Highly suggest to use SDS-proteinase K digestion and alcohol precipitation method to isolated total DNA since proteinase K can release cccDNA from possible covalent link to proteins. In addition, alcohol precipitation can recover non-exclusively both non-cccDNA and cccDNA.
- To avoid the nicking of cccDNA by residual DNase in total DNA preparation, perform cccDNA purification immediately after total DNA isolation, or quick-freeze total DNA preparation immediately and store at –80℃ until cccDNA isolation.
- Total DNA should be dissolved in DNase-free water or TE solution, not in other solvent, otherwise a trial experiment is needed to test the compatibility of this solvent and this kit.

Kit Contents

Component	CAT#:220501-100	CAT#:220502-100
cccDNA Extraction Solution A	100 mL	100 mL
cccDNA Extraction Solution B	-	30 mL
cccDNA Precipitation Solution	-	100 mL

Storage and Handling

Store the kit at room temperature. The kit is stable for at least one year from date of receipt.

Required Materials not Supplied

Unless otherwise indicated, all materials are available through major laboratory supplier.

No	Item
1	bench-top centrifuge with max speed>12,000×g and 4℃
2	1.5mL polypropylene microcentrifuge tube
3	vortex
4	DNase-free water
5	75% Ethanol

Procedural Guidelines

- Use purified total DNA or partially purified cccDNA as starting materials.
- Perform all steps at room temperature (20–25℃) unless otherwise noted.
- Use sterile, disposable DNase-free pipette tips, and tubes.
- Wear disposable gloves while handling reagents and DNA samples to prevent DNase contamination from the surface of the skin.
- Use proper microbiological aseptic techniques when working with DNA.
- Make sure that all materials that come into contact with the kit are compatible with phenol, guanidine isothiocyanate, and chloroform.

Procedures

Section 1: cccDNA Extraction

1. Transfer no more than 200μL total DNA solution (in TE or in DNase-free water) or enzymatic reaction solution to a new 1.5mL microcentrifuge tube.
2. Add DNase-free water to bring up the volume to 200μL. If the volume of the input DNA solution is already 200μL, skip this step.
3. Add 0.6mL cccDNA Extraction Solution A to the microcentrifuge tube, then securely cap the tube and vortex vigorously for two minutes，then proceed directly to one of the following three sections

(Section 2-4) depending on the cccDNA recovery methods chosen.

Section 2: cccDNA recovery by precipitation. The kit CAT#220502-100 contains related reagents. The kit CAT#220501-100 does not.

4. Add 0.2 mL of cccDNA Extraction Solution B to the mixture from step 3, then securely cap the tube and vortex vigorously for two minutes.
5. Centrifuge the sample for 3 minutes at 12,000 × g at 4℃. The mixture separates into a colorless lower phase and a colorless upper phase.
6. Carefully transfer to a new 1.5mL microcentrifuge tube the upper phase which contains the cccDNA.
7. Shake briefly to mix cccDNA Precipitation Solution (it contains some flocculent precipitate) and transfer 0.6 mL of this solution to the upper phase from step 6, then securely cap the tube and vortex the tube vigorously for 30 seconds.
8. Centrifuge the sample for 10 minutes at 12,000 × g at 4℃. A tiny pellet at the bottom of the microtube 's centrifugal side is visible.
9. Carefully discard the supernatant. Make sure the tiny pellet remains at the bottom of the tube.
10. Add 1 mL of 75% ethanol to the pellet, securely cap the tube and inverts the tubes ten times.
11. Centrifuge the sample for 5 minutes at 12,000 × g at 4℃.
12. Carefully discard the supernatant. Make sure the tiny pellet is still at the bottom of the tube.
13. Centrifuge the sample for 30 seconds at 12,000 × g at 4℃.
14. Carefully remove the residual liquid (about 50μL) at the bottom of the tube. Make sure the tiny pellet is still at the bottom of the tube.
15. Open the cap and air dry the tube for 3 minutes.
16. Dissolve the pellet with 20–50 μL of DNase-free water.

Section 3: cccDNA recovery by spin column plasmid DNA purification kits based on DNA-silica membrane binding principle. These kits are available from various vendors.

17. Add the mixture from step 3 to the silica-membrane spin column.
18. Centrifuge the column for 3 minutes at 12,000 × g at 4℃ and discard the pass-through solution.
19. Preform washing and eluting steps according to the protocol supplied by the manufacturer of the spin column plasmid DNA purification kit.

Section 4: cccDNA recovery by magnetic beads plasmid DNA purification kits based on DNA-silica membrane binding principle. These kits are available from various vendors.

20. Add appropriate amount of silica-coated magnetic beads to the mixture from step 3.
21. Preform binding, washing and eluting steps according to the protocol supplied by the manufacturer of the magnetic beads plasmid DNA purification kit.

Section 5: Determine the cccDNA yield

22. Measure the absorbance at 260nm and 280nm of the cccDNA solution. 1 OD at 260nm equals to 50μg DNA/mL. A ratio of A260/A280 above 1.8 is considered pure.
23. Optionally, quantify cccDNA yield using the appropriate dsDNA Assay Kit. Refer to the kit's manual for the protocol.
24. Use it immediately or store at -20℃ for later use.

References

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Limited Product Warranty