Monarch® Plasmid DNA Miniprep Kit Protocol (NEB #T1010)

Overview

The Monarch Plasmid Miniprep Kit (NEB #T1010) was replaced by the Monarch® Spin Plasmid Miniprep Kit (NEB #T1110).

The following protocol is designed for use with the T1010 kit only.

General Guidelines:

Yield and quality of plasmid DNA is affected by plasmid copy number, plasmid size, insert toxicity, host strain, antibiotic selection, growth media and culture conditions. For standard cloning strains of *E. coli*, we recommend using a single colony from a freshly streaked selective plate to inoculate a standard growth media, such as LB (Luria-Bertaini) media. Cultures are typically grown at 37°C and 200–250 RPM in vessels that allow some aeration (Erlenmeyer flasks or culture tubes on a roller drum) and harvested after 12–16 hours as the culture transitions from logarithmic growth to stationary phase. This is the time at which the plasmid DNA content is highest. While cultures in LB often saturate with a final OD_{600} between 3–6, growth to saturation often leads to cell lysis. As a result, plasmid yields and quality are reduced and the likelihood of co-purifying unwanted host chromosomal DNA increases. Use of rich media, such as 2X YT or TB, produces higher biomass in a shorter time period. If chosen for growth, adjustments to the culture times and amount of cells used in the prep should be made to correct for these differences, and to avoid overloading the matrix and reducing DNA yield and quality.

PLASMID	REPLICON	COPY NUMBER	CLASSIFICATION
pUC and its derivatives	pMB1*	> 75	High copy
pBR322 and its derivatives	pMB1	15–20	Low copy
pACYC and its derivatives	p15A	10–12	Low copy
pSC101	pSC101	~5	

*pUC and its derivatives lack the *Rop* gene and contain a point mutation in the RNAII transcript. These changes result in higher copy number during routine growth with many sources reporting levels as high as 500 copies per cell.

Antibiotics for Plasmid Selection

ANTIBIOTIC	CONCENTRATION OF STOCK SOLUTION	STORAGE TEMP.	WORKING CONCENTRATION
Ampicillin	100 mg/ml (H_2O)	–20°C	50–200 µg/ml
Carbenicillin	100 mg/ml (H_2O)	–20°C	20–200 µg/ml
Chloramphenicol	34 mg/ml (ethanol)	–20°C	25–170 µg/ml
Kanamycin	10 mg/ml (H_2O)	–20°C	10–50 µg/ml

ANTIBIOTIC	CONCENTRATION OF STOCK SOLUTION	STORAGE TEMP.	WORKING CONCENTRATION
Streptomycin	10 mg/ml (H_2O)	–20°C	10–50 µg/ml
Tetracycline	5 mg/ml (ethanol)	–20°C	10–50 µg/ml

Buffer Preparation:

Add ethanol to Monarch Plasmid Wash Buffer 2 prior to use (4 volumes of ≥ 95% ethanol per volume of Monarch Plasmid Wash Buffer 2).

- For 50-prep kit add 24 ml of ethanol to 6 ml of Monarch Plasmid Wash Buffer 2
- For 250-prep kit add 120 ml of ethanol to 30 ml of Monarch Plasmid Wash Buffer 2

Always keep all buffer bottles tightly closed when not actively in use.

Protocol:

All centrifugation steps should be carried out at 16,000 x g (~13,000 RPM).

If precipitate has formed in Lysis Buffer (B2), incubate at 30–37°C, inverting periodically to dissolve.

Store Plasmid Neutralization Buffer (B3) at 4°C after opening, as it contains RNase A.

Note: unlike other commercial kits, all wash steps are required.

1. Pellet 1–5 ml bacterial culture (not to exceed 15 OD units) by centrifugation for 30 seconds. Discard supernatant.

   Note: For a standard miniprep to prepare DNA for restriction digestion or PCR, we recommend 1.5 ml of culture, as this is sufficient for most applications. Ensure cultures are not overgrown (12-16 hours is ideal).

2. Resuspend pellet in 200 µl Plasmid Resuspension Buffer (B1) (pink). Vortex or pipet to ensure cells are completely resuspended. There should be no visible clumps.

3. Lyse cells by adding 200 µl Plasmid Lysis Buffer (B2) (blue/green). Invert tube immediately and gently 5–6 times until color changes to dark pink and the solution is clear and viscous. Do not vortex! Incubate for one minute.

   Note: Care should be taken not to handle the sample roughly and risk shearing chromosomal DNA, which will co-purify as a contaminant. Avoid incubating longer than one minute to prevent irreversible plasmid denaturation.

4. Neutralize the lysate by adding 400 µl of Plasmid Neutralization Buffer (B3) (yellow). Gently invert tube until color is uniformly yellow and a precipitate forms. Do not vortex! Incubate for 2 minutes.

   Note: Be careful not to shear chromosomal DNA by vortexing or vigorous shaking. Firmly inverting the tube promotes good mixing, important for full neutralization.

5. Clarify the lysate by spinning for 2–5 minutes at 16,000 x g.

   Note: Spin time should not be less than 2 minutes. Careful handling of the tube will ensure no debris is transferred and the 2 minute recommended spin can be successfully employed to save valuable time. For culture volumes > 1 ml, we recommend a 5 minute spin to ensure efficient RNA removal by RNase A. Also, longer spin times will result in a more compact pellet that lower the risk of clogging the column.

To save time, spin for two minutes only.

6. Carefully transfer supernatant to the spin column and centrifuge for 1 minute. Discard flow-through.

To save time, spin for 30 seconds, instead of 1 minute.

If using a vacuum manifold instead of centrifugation, insert the column into a manifold and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.*

7. Re-insert column in the collection tube and add 200 µl of Plasmid Wash Buffer 1. Plasmid Wash Buffer 1 removes RNA, protein and endotoxin. (Add a 5 minute incubation step before centrifugation if the DNA will be used in transfection.) Centrifuge for 1 minute. Discarding the flow-through is optional.

   Note: The collection tube is designed to hold 800 µl of flow-through fluid and still allow the tip of the column to be safely above the top of the liquid. Empty the tube whenever necessary to ensure the column tip and flow-through do not make contact.

   To save time, spin for 30 seconds, instead of 1 minute.

   If using a vacuum manifold, add 200 µl of Plasmid Wash Buffer 1 and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

   ** Make sure to follow the manifold manufacturer's instructions to set-up the manifold and connect it properly to a vacuum source.*

8. Add 400 µl of Plasmid Wash Buffer 2 and centrifuge for 1 minute.

   When using a manifold add 400 µl of Plasmid Wash Buffer 2 and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

9. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column has not come into contact with the flow-through. If there is any doubt, re-spin the column for 1 minute before inserting it into the clean microfuge tube.

   If using a vacuum manifold: Since vacuum set-ups can vary, a 1 minute centrifugation is recommended prior to elution to ensure that no traces of salt and ethanol are carried over to the next step.

10. Add ≥ 30 µl DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.

    Note: Nuclease-free water (pH 7–8.5) can also be used to elute the DNA. Delivery of the Monarch DNA Elution Buffer should be made directly to the center of the column to ensure the matrix is completely covered for maximal efficiency of elution. Additionally, yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated as a result of dilution. For larger plasmids (≥ 10 kb), heating the DNA Elution Buffer to 50°C prior to eluting and extending the incubation time after buffer addition to 5 minutes can improve yield.