Plasmid Miniprep Kit

User Guide

FOR RESEARCH USE ONLY

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1. Introduction

Quintech Plasmid Miniprep Kit is designed for fast and efficient purification of plasmid DNA from 1 to 4 ml of *E. coli* culture. The mini column has a plasmid DNA binding capacity of 50 µg. The yield from 1 ml culture is typically around 8 to 12 µg.

Our proprietary DNA binding system results in a highly efficient and reversible binding of DNA to the ezBind mini column, with proteins and other impurities removed by wash buffer. Nucleic acids are then eluted with sterile water or elution buffer.

The purified DNA is ready for downstream applications such as cloning and subcloning, RFLP, library screening, *in vitro* translation, sequencing and transfection of robust cells such as HEK293 cells.

2. Kit Components & Safety Information

Catalog#	QT100-1101S SAMPLE	QT100-1102 50 Preps	QT100-1101 250 Preps
Preps	6	50	250
ezBind Mini Columns	6	50	250
Buffer BK	3.5 ml	30 ml	150 ml
Buffer A1	1.5 ml	20 ml	70 ml
Buffer A2	1.5 ml	25 ml	70 ml
Buffer A3^{+}	2.0 ml	30 ml	100 ml
Buffer W1	3.1 ml	30 ml	135 ml
Buffer W2*	3.5 ml with ethanol added	12 ml	3 x 24 ml
Buffer EB	500µl	10 ml	30 ml
RNase A (20 mg/ml)	Added to Buffer A1	2.0mg (100 µL)	7.0 mg (350 µL)
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*48 ml (50 preps) or 96 ml (250 preps) 95-100% ethanol to Buffer W2 before use.

$^{+}$Buffer A3 contains acidic acid. Please handle with gloves and protective eyewear. Buffer A3 and W1 contains chaotropic salts, which may form reactive compounds when in contact with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

3. Storage

Quintech Plasmid Miniprep Kit should be stored dry at room temperature. Product performance and quality is maintained for kits stored up to 12 months at room temperature. For longer storage, kits can be stored at 2-8°C. Should any precipitate be observed in the buffers, redissolve at 37°C. RNase is stable for more than 6 months when stored at room temperature. Once resuspended, store at 4°C.

4. Supplies Needed

Materials to be supplied by user:
1.5 ml microcentrifuge tubes
95-100% ethanol
High speed microcentrifuge (13,000 rpm)
55-60°C waterbath
Vacuum manifold

Materials to be prepared:
Spin down RNase A vial briefly. Add RNase A solution to buffer A1 t and mix well before use. Store at 4°C

48 ml (50 preps) or 96 ml (250 preps) 95-100% ethanol to each bottle of Buffer W2 before use

Buffer A2 may form precipitate below room temperature. It is critical to warm up the buffer at 50℃ to dissolve the precipitate before use

Keep the cap tightly closed for Buffer A2 after opening

Perform all steps including centrifugations at room temperature

5. Plasmid Miniprep: Spin Protocol

1. Inoculate **1-5 ml** LB containing appropriate antibiotic with a fresh colony from a freshly streaked selective plate. Incubate at 37°C for 14-16 hours with vigorous shaking.

   Note:
   - Prolonged incubation (>16 hours) is not recommended since the *E.coli* starts to lyse and the plasmid yields may be reduced
   - Do not grow the culture directly from the glycerol stock
   - This protocol is optimized for *E. coli* strain cultured in LB medium. When using TB or 2xYT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD_{600}). Buffers need to be scaled up proportionally if larger volume of cultures are being processed

2. Add **500 µl Buffer BK** into the spin column, let it stand until step 5.

3. Harvest the bacterial culture by centrifugation for 1 min at 10,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residual medium completely.

   Note: Residual medium results in poor cell lysis, lowering DNA yield. Loosen pellet after centrifugation in step 6

4. Add **250 µl Buffer A1** (Add RNase A to **Buffer A1** before use) and completely resuspend bacterial pellet by vortexing or pipetting.

   Note: Complete resuspension is critical for bacterial lysis and lysate neutralization

5. Add **250 µl Buffer A2**, mix gently by inverting the tube 10 times (do not vortex) and incubate at room temperature for 5 minutes.

   Note:
   - Do not incubate for more than 5 min
   - Buffer A2 precipitates below room temperature. Warm up at 50°C to dissolve precipitation before use
   - Spin the column with BK at 13 k rpm for 2 mins

6. Add **350 µl Buffer A3**, mix completely by inverting and shaking the vial for 5 times and sharp hand shaking for 2 times.

   Note:
   - Incubate the lysate in ice for 1 min to improve yield
   - It is critical to mix the solution well. If the mixture appears conglobated, brownish or viscous, more mixing is required to completely neutralize the solution

7. Centrifuge the lysate at 13,000 rpm for 10 min at room temperature

   Note: If the lysate does not appear clear, reverse tube angle and centrifuge for an additional 5 min. Transfer clear lysate to DNA column

8. Carefully transfer the clear lysate into a DNA column with a collection tube. Incubate the supernatant in column for at least 2 min before spinning at 13,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube.

9. **Optional:** Add **500 µl Buffer W1** into the spin column, centrifuge at 13,000 rpm for 1 min. Remove the spin column from the tube and discard the flow-through. Put the column back to the collection tube.

   Note: Buffer W1 is recommended for *end A+* strains such as HB101, JM101, TG1 or their derived strains

10. Add **500 µl Buffer W2** *(Add ethanol to Buffer W2 before use)* into the spin column, centrifuge at 13,000 rpm for 1 min at room temperature. Remove the spin column from the tube and discard the flow-through. Repeat step 9 to improve the recovery.

11. Reinsert the spin column, **with the lid open,** into the collection tube and centrifuge for 2 min at 13,000 rpm.

    Note: Residual ethanol can be removed more efficiently with the column lid open. It is critical to remove residual ethanol completely.

    Carefully transfer the spin column into a sterile 1.5 ml tube and add **50-100 µL (> 50 µL) sterile ddH20** or **Buffer EB** to the center of the column and let it stand for 2 min. Centrifuge at 13,000 rpm for 1 min to elute the DNA. Reload the eluent into the column for a second elution and spin at same speed for 2 min.

    Note: If ddH2O is applied, make sure that the pH is no less than 7.0 (7.0-8.5 is preferred). NaOH can be used to adjust the pH of ddH2O

The DNA is now ready for downstream applications such as cloning and subcloning, RFLP, library screening, *in vitro* translation, sequencing, transfection of robust cells such as HEK293 cells.

The DNA concentration can be calculated as follows:
Concentration (µg/ml) = OD_{260nm} x 50 x dilution factor.

6. Plasmid Miniprep: Vacuum Protocol

1. Prepare the vacuum manifold according to the manufacturer's instruction. Attach the spin column to the manifold.

2. Carry out steps 1to 7 of section 6 (spin protocol).

3. Carefully transfer the clear lysate to the DNA column and turn on the vacuum to allow the lysate to pass through the column.

4. **Optional**: Add **500 µl Buffer W1** into the spin column and allow the lysate to pass through the column by vacuum.

   Note: Buffer W1 is recommended for isolating plasmid from *endA*+ strains such as HB101, JM101, TG1 or their derived strains. It is not necessary for isolating DNA from *endA*- strains such as Top 10 and DH5a.

5. Add **650 µl** of **Buffer W2** to the column and allow the vacuum to draw the liquid through the manifold. Turn off the vacuum. Repeat step 5 to improve the recovery.

6. Transfer the column, **with the lid open**, to a 1.5 ml collection tube and centrifuge at 13,000 rpm for 2 min.

   Carefully transfer the spin column into a clean 1.5 ml tube and add **50-100 µL** (**>50 µL) sterile ddH20** or **Buffer EB** into the column and let it stand for 2 min. Centrifuge at 13,000 rpm for 1 min to elute the DNA. Reload the eluent into the column for a second elution.

The DNA is now ready for downstream applications such as cloning and subcloning, RFLP, library screening, *in vitro* translation, sequencing, transfection of robust cells such as HEK293 cells.

The DNA concentration can be calculated as follows:
Concentration (µg/ml) = OD_{260nm} x 50 x dilution factor.

7. Purification of Low Copy Number Plasmid and Cosmid

The yield of low copy number plasmid is normally around 0.1–1 µg/ml of overnight culture.

For isolating low copy number or medium copy number plasmid DNA, use the following guideline:

- Culture volume: Use **2 x volumes** of the high copy number culture
- Use **2 x volumes** of the **Buffer A1**, **Buffer A2** and **Buffer A3**
- Use the **same volume** of wash buffer **(Buffer W2)** and elution buffer **(Buffer EB)**

8. Purification of Plasmid (>12kb)

For isolating plasmid DNA > 12 kb, use the following guideline:

- Culture volume: Use **2 x volumes** of the high copy number culture
- Use **2 x volumes** of the **Buffer A1**, **Buffer A2** and **Buffer A3**
- Use the **same volume** of wash buffer **(Buffer W2)** and elution buffer **(Buffer EB)**
- Pre-warm **Buffer EB** at 65-70°C and let the column stand for 5 min after adding elution buffer (**Buffer EB)**

9. Troubleshooting Guide

Problem	Possible Reason	Recommended Solution
Low DNA yield	Poor cell lysis	Resuspend pellet thoroughly by vortexing and pipetting prior to adding Buffer A2. Ensure that Buffer A2 is fresh
	Bacterial culture overgrown or not fresh	Grow bacteria for 12-16 hours. Spin down cultures and store the pellet at -20 °C if the culture is not purified the same day. Do not store culture at 4°C over night.
	Low copy-number plasmid	Increase culture volume and the volume of Buffer A1, B1, N1 as instructed on page 12
No DNA yield	Plasmid lost in host *E. coli*	Prepare fresh culture
Genomic DNA contamination	Over-time incubation after adding Buffer A2	Do not vortex or mix aggressively after adding Buffer A2. Do not incubate more than 5 min after adding Buffer A2
RNA contamination	RNase A not added to Buffer A1	Add RNase A to Buffer A1
Plasmid DNA floats out of well while running in agarose gel, DNA does not freeze or smell of ethanol	Ethanol traces were not completely removed from column during wash step	Make sure that no ethanol residue remains in the silicon membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary

10. Ordering Information

Catalog #	Description
QT100-1101	Quintech Plasmid Miniprep Kit, 250 preps Includes 250 ezBind mini columns, reagents and user guide
QT100-1102	Quintech Plasmid Miniprep Kit, 50 preps Includes 50 ezBind mini columns, reagents and user guide
Kit Components	
QT100-8101C	ezBind Mini Columns, 250 preps
QT100-8102C	ezBind Mini Columns, 50 preps
QT100-8201BK	Buffer BK, 150 ml, 250 preps
QT100-8202BK	Buffer BK, 30 ml, 50 preps
QT100-8203A1	Buffer A1, 70 ml, 250 preps
QT100-8204A1	Buffer A1, 20 ml, 50 preps
QT100-8205A2	Buffer A2, 70 ml, 250 preps
QT100-8206A2	Buffer A2, 25 ml, 50 preps
QT100-8207A3	Buffer A3, 100 ml, 250 preps
QT100-8208A3	Buffer A3, 30 ml, 50 preps
QT100-8209W1	Buffer W1, 135 ml, 250 preps
QT100-8210W1	Buffer W1, 30 ml, 50 preps
QT100-8211W2	Buffer W2, 24 ml, 3, 250 preps
QT100-8212W2	Buffer W2, 12 ml, 50 preps
QT100-8213EB	Buffer EB, 30 ml, 250 preps
QT100-8214EB	Buffer EB, 10 ml, 50 preps
QT100-8301RA	RNase A (20mg/ml), 7.0 mg, 250 preps
QT100-8302RA	RNase A (20mg.ml), 2.0 mg, 50 preps

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Simplifying Sample Purification

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