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SPEEDTOOLS PLASMID DNA PURIFICATION KIT

for small-scale preparation of pure plasmidic DNA (1 - 5 ml bacteria culture)

Instructions for Use

(Ref. 21.220M/1/2)

PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.

1. BASIC PRINCIPLE

SPEEDTOOLS PLASMID DNA PURIFICATION KIT is designed for a rapid and easy preparation of highly pure plasmid DNA (mini preps). Based on an alkaline lysis procedure, followed by binding of the plasmid DNA to a silica membrane column, and elution of the pure plasmid DNA.

In a first step pelleted bacteria from 1 - 5 ml culture are suspended in Buffer R and subjected to alkaline/SDS lysis procedure by addition of Buffer L. The resulting lysate is treated with Buffer N, which neutralizes the lysate and creates the appropriate conditions for binding of plasmid DNA to the silica membrane of the Binding Column. Precipitated proteins, genomic DNA, and cell debris are then pelleted by centrifugation. The supernatant containing soluble components as plasmid DNA is loaded onto the column and washed with Wash Buffer 2. This single washing procedure eliminates contaminants like salts, metabolites, and soluble macromolecular cellular components. If host strains with high levels of nucleases are used, an additional washing step with preheated Wash Buffer 1 is recommended. Additional washing with Wash Buffer 1 will also increase the reading length of automated fluorescent DNA sequencing reactions. Pure plasmid DNA is finally eluted under low ionic strength conditions with slightly alkaline Buffer E.

2. KIT SPECIFICATIONS

With the **SPEEDTOOLS PLASMID DNA PURIFICATION KIT** method, plasmid DNA is isolated and purified from cultured cells. The protocol, as well as all the buffers have been optimized to provide high yield and purity of the isolated plasmid DNA. The hands-on-time for the whole procedure is reduced to a minimum.

The kit is suitable for the isolation and purification of any plasmid, the size range for most effective purification is < 10 Kb. Good results have also been obtained with 20 Kb and bigger size plasmids although the obtained yield is reduced.

The obtained plasmid DNA is ready to use for a broad panel of downstream applications like PCR, restriction enzyme digestion, labeling, cloning, DNA sequencing reactions…

Amount of Starting Material	Yield	Time for preparation
1 - 5 ml of bacterial cultures	Up to 60 μg	25 min/18 preps

3. KIT CONTENTS

SPEEDTOOLS PLASMID DNA PURIFICATION KIT			
Reagents	**10 Preps Ref. 21.220M**	**50 Preps Ref. 21.221**	**250 Preps Ref. 21.222**
BUFFER R Resuspension Buffer	3 ml	15 ml	5 x 15 ml
BUFFER L (concentrated) Lysis Buffer	3 ml	15 ml	5 x 15 ml
BUFFER N Neutralization Buffer	4 ml	20 ml	5 x 20 ml
Wash Buffer 1 Washing Solution 1	6 ml	30 ml	5 x 30 ml
Wash Buffer 2 Washing Solution 2	2,5 ml	12 ml	5 x 12 ml
Buffer E Elution Buffer	2,6 ml	13 ml	5 x 13 ml
RNase A (lyophilized)	1,2 mg	6 mg	5 x 6 mg
BIOTOOLS Binding Column	10	50	5 x 50
Collection Tubes (2 ml)	10	50	5 x 50

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Molecular Grade Ethanol 96-100%
- Microcentrifuge and microtubes of 1.5
- Vortex
- Automatic pipettes and tips
- Lab Coat, gloves ang goggles

5. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

Attention: Buffer N and Wash Buffer 1 contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste. Wear gloves and goggles!

Store buffers at room temperature (18-25ºC) until expiration date printed on the Kit label.

Always keep buffer bottles tightly closed, especially if buffers are preheated during the preparation.

Sodium dodecil sulphate (SDS) in Buffer L may precipitate if stored at temperatures below 20º. If a precipitate is observed in Buffer L, incubate the bottle at 30º- 40º for several minutes and mix well.

Before starting any protocol prepare the following:

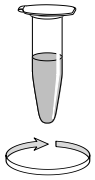
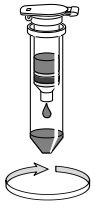
- Add 1ml of Buffer R to the RNase A vial and vortex. Transfer the solution back into de Buffer R bottle and mix thoroughly. Indicate date of RNase A addition. Store Buffer R containing RNase A at 4º.

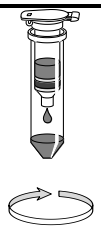
- Add the indicated volume of 96 - 100% molecular grade ethanol to Wash Buffer 2

	10 Preps Ref. 21.220M	**50 Preps Ref. 21.221**	**250 Preps Ref. 21.222**
Wash Buffer 2	2,5 ml Add 10 ml molecular grade ethanol	12 ml Add 48 ml molecular grade ethanol	5 x 12 ml Add 48 ml molecular grade ethanol to each bottle

6. INSTRUCTION FOR USE

Before starting with the protocol prepare the solutions according to section 5

STEP	DESCRIPTION		
1	CULTIVATE AND HARVEST BACTERIAL CELLS Use 1- 5 ml of a saturated *E. coli* LB culture, pellet cells in a standard benchtop microcentrifuge for 30s at 11,000 x g. Discard the supernatant and remove as much of the liquid as possible.		11,000 x g 30 s
2	CELL LYSIS Add **250 µl** of **Buffer R**. Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain before addition of Buffer L. **Attention:** Check Buffer L for precipitated SDS prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30º-40º until precipitate is dissolved completely. Mix thoroughly and cool buffer down to room temperature. (18º - 25º) Add **250 µl Buffer L**. Mix gently by inverting the tube. Do not vortex to avoid shearing of genomic DNA. Incubate at room temperature for up to 5 min or until lysate appears clear. Add **300 µl Buffer N**. Mix thoroughly by inverting the tube until blue samples turn colorless completely!. Do not vortex to avoid shearing of genomic DNA! Make sure to neutralize completely to precipitate all protein and chromosomal DNA. Lyse control should turn completely colorless without any traces of blue.		+ 250 µl BUFFER R Resuspend + 250 µl BUFFER L RT 5 min + 300 µl BUFFER N Mix
3	CLARIFICATION OF LYSATE Centrifuge for **5 min** at **11,000 x g** at room temperature. Repeat this step in case the supernatant is not clear		5 - 10 min 11,000 × g
4	BIND DNA Place a BIOTOOLS Binding Column in a **collection tube (2 ml)** and decant the supernatant from step 3 or pipette a **maximum of 750 µl** of the supernatant onto the column. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the column back into the collection tube. Repeat this step to load the remaining lysate.		Load supernatant Incubate RT 1 min 1 min, 10,000 × g

5	WASH SILICA MEMBRANE **Recommended:** If plasmid DNA is prepared from host strains containing high levels of nucleases it is strongly recommended performing and additional washing step with **500 µl Wash Buffer 1**, preheated to 50ºC, and centrifuge for **1 min** at **11,000 x g** before proceeding with Wash Buffer 2. Additional washing with Wash Buffer 1 will also increase the reading length of DNA sequencing reactions and improve the performance of critical enzymatic reactions. Add **600 µl Wash Buffer 2** (Supplemented with molecular grade ethanol, see section 5). Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the column back into the empty collection tube.		Optional: + 500 µl Wash Buffer 1 1 min, 11,000 × g + 600 µl Wash Buffer 2 1 min, 11,000 x g
6	DRY SILICA MEMBRANE Centrifuge for **2 min** at full speed **11,000 x g** and discard the collection tube.		2 min, 11,000 × g
7	ELUTE DNA Place the binding column in a new 1.5 ml microcentrifuge tube (not provided) and add 50 µl **Buffer E**. Dispense buffer directly onto the silica membrane. **Incubate** at room temperature for **1 min**. Centrifuge **1 min at 11,000 x *g***. The eluate contains your pure plasmid DNA.		+ 50 µl BUFFER E Incubate 1 min 1 min, 11,000 × g

7. TROUBLESHOOTING

Problem	Possible cause and suggestions
Incomplete lysis of bacterial cells	***Cell pellet not properly resuspended*** • It is essential that the cell pellet is completely resuspended prior to lysis. No cell clumps should be visible before addition of Buffer L ***SDS in Buffer L precipitated*** • SDS in buffer L may precipitate upon storage. If a precipitate is formed, incubate Buffer L at 30º-40º for 5 min and mix well. ***Too many bacterial cells used*** • We recommend LB as optimal growth medium. When using very rich media like TB, the cell density of the cultures may become too high

Poor plasmid yield	***Incomplete lysis of bacterial cells*** • See possible cause and suggestions above ***Suboptimal precipitation of SDS and cell debris*** • Precipitation of SDS and cell debris will be slightly more effective when centrifuging at 4ºC instead of room temperature ***No or insufficient amounts of antibiotic used during cultivation*** • Cells carrying the plasmid of interest may become overgrown by non-transformed cells, when inadequate levels of the appropriate antibiotics are used. Add appropriate amounts of freshly prepared stock solutions to all media; both solid and liquid ***Bacterial culture too old*** • Do not incubate cultures for more than 16h at 37ºC under shaking. We recommend LB as the optimal growth medium; however when using very rich media like TB, cultivation time should be reduced to < 12 h. ***Suboptimal elution conditions*** • If possible, use a slightly alkaline elution buffer like Buffer E. If nuclease-free water is used, check the pH of the water. Elution efficiencies drop drastically with buffers < pH 7 ***No high copy-number of plasmid was used*** • If using low copy-number plasmids, the culture volumes should be increased to at least 5 ml
No plasmid yield	***Reagents not applied properly*** • Add indicated volume of 96-100% molecular grade ethanol to Wash Buffer 2 and mix thoroughly (see section 5) ***Nuclease-rich host strain used*** • Especially when working with nuclease-rich strains, keep plasmid preparations on ice or frozen in order to avoid DNA degradation • If using nuclease-rich strains be sure to perform the optional Wash Buffer 1 washing step. Optimal endonuclease removal can be achieved by incubating the membrane with preheated Wash Buffer 1 (50ºC) for 2 min before centrifugation. ***Inappropriate storage of plasmid DNA*** • Quantitate DNA directly after preparation, for example, by agarose gel electrophoresis. Store plasmid DNA dissolved in water at < -18ºC or at < 5ºC when dissolved in TE Buffer.
Poor plasmid quality	***Nicked plasmid DNA*** • Cell suspension was incubated with alkaline Buffer L for more than 5 min ***Genomic DNA contamination*** • Cell lysate was vortexed or mixed too vigorously after addition of Buffer L. Genomic DNA was sheared and thus liberated ***Smeared plasmid bands on agarose gel*** • Especially when working with nuclease-rich strains, keep plasmid preparations on ice or frozen in order to avoid DNA degradation • If using nuclease-rich strains be sure to perform the optional Wash Buffer 1 washing step. Optimal endonuclease removal can be achieved by incubating the membrane with preheated Wash Buffer 1 (50ºC) for 2 min before centrifugation.

Suboptimal performance of plasmid DNA in enzymatic reactions	***Carry-over of ethanol*** • Make sure to centrifuge ≥ 1 min at 11,000 x g in order to achieve complete removal of ethanolic Wash Buffer 2 ***Elution of plasmid DNA with TE Buffer*** • EDTA may inhibit sequencing reactions. Repurify plasmid DNA and elute with Buffer E or water. Alternatively, the eluted plasmid DNA can be precipitated with molecular grade ethanol and redissolved in Buffer E or water. ***No additional washing with Wash Buffer 1 performed*** • Additional washing with 500 µl of Wash Buffer 1 before washing with Wash Buffer 2 will increase the reading length of sequencing reactions. ***Not enough DNA used for sequencing reaction*** • Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions ***Plasmid DNA prepared from too much bacterial cell material*** • Do not use more than 3 ml of a saturated *E. Coli* culture if preparing plasmid DNA for automated fluorescent DNA sequencing

8. ORDERING INFORMATION

SPEEDTOOLS KIT	10 PREPS	50 PREPS	250 PREPS
SPEEDTOOLS **DNA EXTRACTION** KIT	Ref. 21.130M	Ref. 21.131	Ref. 21.132
SPEEDTOOLS **TISSUE DNA EXTRACTION** KIT	Ref. 21.135M	Ref. 21.136	Ref. 21.137
SPEEDTOOLS **RNA VIRUS EXTRACTION** KIT	Ref. 21.140M	Ref. 21.141	Ref. 21.142
SPEEDTOOLS **FOOD DNA EXTRACTION** KIT	Ref. 21.175M	Ref. 21.176	Ref. 21.177
SPEEDTOOLS **PLANT DNA EXTRACTION** KIT	Ref. 21.170M	Ref. 21.171	Ref. 21.172
SPEEDTOOLS **TOTAL RNA EXTRACTION** KIT	Ref. 21.210M	Ref. 21.211	Ref. 21.212
SPEEDTOOLS **PCR CLEAN-UP** KIT	Ref. 21.200M	Ref. 21.201	Ref. 21.202
SPEEDTOOLS **PLASMID DNA PURIFICATION** KIT	Ref. 21.220M	Ref. 21.221	Ref. 21.222

9. PRODUCT USE RESTRICTION AND WARRANTY

1. Product for research purposes only.
2. The kit includes documentation stating specifications and other technical information. Follow the instructions in order to obtain highly pure plasmid DNA.
3. The components of the Kit were tested according to ISO 9001-2001 and EN 13485-2003.
4. The user is responsible to validate the performance of the Kit for any particular use, since the performance characteristics of the kit have not been validated for any specific application. The Kit may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA'88 regulations in the U.S. or equivalents in other countries.
5. BIOTOOLS warrants to meet the stated specifications of the kit. Any product not fulfilling the specifications included in the product sheet will be replaced.
6. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.
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