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SPEEDTOOLS PLASMID DNA PURIFICATION KIT

for small-scale preparation of pure plasmidic DNA (0.5 - 2 ml bacteria culture)

Instructions for Use

(Cat. No.21.221)

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 0.5 - 2 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 ethanolic Solution W. This single washing procedure eliminates contaminants like salts, metabolites, and soluble macromolecular cellular components. 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, capillary sequencing.

Amount of Starting Material	**Yield**	**Time for preparation**	**Ratio A_{260}/A_{280}**
0.5 - 2 ml of bacterial cultures	Up to 20 μg (2 ml culture)	< 15 min/4 preps	1.8-2.1

3. KIT CONTENTS

SPEEDTOOLS PLASMID DNA PURIFICATION KIT		
	50 Preps Cat. No. 21.221	250 Preps Cat. No. 21.222
BUFFER R Resuspension Buffer	15 ml	5X (15 ml)
BUFFER L (concentrated) Lysis Buffer	15 ml	5X (15 ml)
BUFFER N Neutralization Buffer	15 ml	5X (15 ml)
Solution W Washing Solution	12 ml	5X (12 ml)
Buffer E Elution Buffer	10 ml	5X (10 ml)
BIOTOOLS Binding Column	50	5X (50)
Collecting Tubes (2 ml)	50	5X (50)
Collecting Tubes (1.5 ml)	50	5X (50)
Manual	1	1

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Ethanol 96-100%
- Microcentrifuge and microtubes of 1.5 and 2 ml
- Vortex
- Automatic pipettes and tips

5. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

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

Before starting any protocol prepare the following reagent:

- The Washing Solution (Solution W) is delivered as a concentrate. To prepare the final Washing Solution add 48 ml of 96-100% ethanol.

Make sure that all components are stored at room temperature if there are any precipitates in the provided reagents dissolve them by carefully warming up to room temperature and mix by inversion.

6. SAFETY INSTRUCTIONS

The following components of the **SPEEDTOOLS PLASMID DNA PURIFICATION KIT** contain hazardous contents. *Wear gloves and goggles and follow the safety instructions given in this section*. Avoid any skin contact. In case of contact, immediately flush eyes or skin with a large amount of water.

Reagent	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
Buffer N	Acetic acid Guanidine	Xn Harmful C Corrosive	Harmful by inhalation, in contact with skin and if swallowed Contact with acids liberates very toxic gas Causes burns Keep out of the reach of children Keep away from food, drink and animal feeding stuffs Do not breath gas/fumes/vapour/spray In case of contact with eyes, rinse immediately with plenty of water and seek medical advice In case of accident or if you feel unwell, seek medical advice	R 20/21/22 R32-R34	S2-S13-S23-S26-S45

7. INSTRUCTION FOR USE

Before starting with the protocol prepare Solution W (see section 5).

STEP	DESCRIPTION		
1	**HARVEST AND SUSPENSION OF BACTERIAL CELLS** Transfer 0.5 ml up to 2 ml of the overnight *E. coli* culture into a 2 ml micro centrifuge tube. Centrifuge for **1 min at maximum speed** to pellet the cells. Remove the supernatant as completely as possible. Resuspend the cell pellet in **250 µl of Buffer R** completely by vortexing or by pipetting up and down. No cell pellet or clumps should be visible.		*HARVEST CELLS* *1 min, max speed* + 250 µl Buffer R
2	**CELL LYSIS** Do not perform the lysis step for more than 5 min. Add **250 µl of Buffer L** close the tube and **mix carefully by inverting the tube** five times. **NOTE:** *to mix the suspension with the lysis buffer do not vortex the tube. This step is critical for the separation of bacterial chromosomal DNA from plasmid DNA. Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. Sheared chromosomal DNA is not precipitated by NaOH/SDS and contaminates the plasmid DNA.*		+ 250 µl BUFFER L Mix
3	**CLARIFICATION OF THE LYSATE** Add **250 µl of Buffer N** and mix gently, but thoroughly, by shaking the tube 4-6 times. Centrifuge for 5 min at **full speed** 13,500-16,500 x *g*.		250 µl BUFFER N *5 min, 13,500 × g*
4	**BIND DNA** For each preparation, take one **BIOTOOLS Binding Column** placed it into a 2 ml Collecting Tube. Transfer the clarified supernatant onto the column. Incubate 1 min and centrifuge **1 min at 10,000 x g**. **Discard the flow-through**.		Load the column Incubate RT 1 min *1 min, 10,000 × g*
5	**WASH SILICA MEMBRANE** Place the column into the collecting tube and add **750 µl Solution W**. Centrifuge **1 min at 10,000 x *g***. **Discard flow-through** and reinsert the column into the empty tube.		750 µl SOLUTION W *1 min, 10,000 × g*
6	**DRY SILICA MEMBRANE** Centrifuge for **3 min** at full speed **13,500-16,500 x *g*** to complete removal of residual ethanol.		*3 min, 13,500 × g*
7	**ELUTE HIGHLY PURE DNA** Place the binding column in a new 1.5 ml collecting tube and add 50 -100 µl **elution Buffer E**. Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge **1 min at 10,000 x *g***. The eluate contains your pure plasmid DNA. **NOTE:** *To* ***increase the final DNA yield*** *use* ***higher volume of Buffer E*** *and* ***increase the incubation time with the elution buffer*** *up to 10 min. In order* ***to increase the DNA concentration elute in smaller volume*** *(the lowest recommended elution volume is 30 µl).* *For in vitro transcription application please elute plasmid DNA in* ddH_2O		+ 50-100 µl BUFFER E Incubate 1 min *1 min, 10,000 × g*

8. TROUBLESHOOTING

Problem	Possible cause and suggestions
Low yield of plasmid DNA	***Incorrect Wash Solution*** • Prepare the Wash Solution as described in section 5 of the manual. Store the washing solution firmly close. ***Poor Elution DNA*** • Add the Elution Buffer E directly on the center of the membrane. ***Conditions for bacterial cultures are not optimal*** • Change the conditions for bacterial growth (media, time, others).
Additional band bellow the supercoiled plasmid DNA band	***Denaturated supercoiled plasmid DNA band*** • Incorrect incubation with the lysis buffer. Increased incubation time with lysis buffer L can cause denaturation of supercoiled plasmid DNA.
Contamination of the plasmid DNA with chromosomal DNA	***The sample was mixed too vigorously during the lysis step*** • Follow exactly the protocol. Do not vortex at this step, mix the samples only by inverting the tubes carefully. ***Bacteria overgrown*** • Reduce the time for growing the bacterial culture
Problems because of poor cleavage by restriction endonucleases or for other applications	***Contamination of the final plasmid DNA with salt components*** • Wash the plasmid DNA bound on the binding column as described ***Contamination of the final DNA with ethanol*** • Keep the given centrifugation time, extend it if necessary. ***Contamination of the final DNA with carryover from the column*** • To spin down particles or contaminants arriving from the column centrifuge the eluted plasmid DNA at full speed for 1 min. Take your plasmidic DNA from the upper fraction because the contaminants will be at the bottom of the tube.

8. ORDERING INFORMATION

SPEEDTOOLS PLASMID DNA PURIFICATION KIT	Cat. No. 21.221	50 preps
SPEEDTOOLS PLASMID DNA PURIFICATION KIT	Cat. No. 21.222	250 preps

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.
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