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peqGOLD
Fastfilter Plasmid Maxi Kit

– Instruction Manual –

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Content

Introduction 3

Kit Content 4

Storage 4

Before Starting 5

peqGOLD Fastfilter Plasmid Maxiprep Protocol 6

Yield and Quality of DNA 9

Ordering Information 10

Troubleshooting Tips 11

Introduction

The peqGOLD family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is the proprietary HiBind® matrix that avidly, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The peqGOLD Fastfilter Plasmid Maxiprep Kit combines the power of HiBind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality DNA. The HiBind® Maxi-columns facilitate the binding, washing, and elution steps thus enabling multiple samples to be simultaneously processed. This kit also include a special filter cartridge, which replaces the centrifugation step following alkaline lysis. Following lysis the DNA is bound to the silica membrane and contaminants are removed with a simple wash step. Yields vary according to plasmid copy number, *E.coli* strain, and conditions of
growth, but up to 1.5 mg of high quality plasmid can be purified from overnight culture. The product is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations.

Kit Content

peqGOLD Fastfilter Plasmid Maxi Kits	5 Purifications	20 Purifications	40Purifications
Order Number	12-6924-01	12-6924-02	12-6924-03
Components			
HiBind®-Maxi Column	5	20	40
Filter syringe	5	20	40
Solution I	60 ml	220 ml	440 ml
Solution II	60 ml	220 ml	440 ml
Neutralisation Buffer	60 ml	220 ml	440 ml
GPS Buffer	55 ml	210 ml	410 ml
Buffer HB	55 ml	210 ml	410 ml
Buffer GBT	55 ml	210 ml	410 ml
Maxi DNA Wash buffer	2 x 40 ml	3 x 60 ml	6 x 60 ml
RNase A	250 µl	800 µl	2 x 800 µl
Elution buffer	20 ml	80 ml	2 x 80 ml
Instruction Booklet	1	1	1

Storage

All peqGOLD Plasmid isolation components are guaranteed for at least 12 months from the date of purchase when stored as follows: RNase A, Solution I (once RNase A is added) at 4°C, all other material at 22-25°C.

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

! Add vial of RNase A to bottle of Solution I provided. Store at 4°C.

! Store solution II tightly capped when not in use..

! DNA wash buffer is to be diluted with absolute ethanol as follows:

Kit	Dilution
Kit 12-6924-01	Add 90 ml 100 % EtOH to 40 ml Wash buffer
Kit 12-6924-02	Add 140 ml 100 % EtOH to 60 ml Wash buffer
Kit 12-6924-03	Add 140 ml 100 % EtOH to 60 ml Wash buffer

Store diluted wash buffer at room temperature.

! All steps must be carried out at room temperature.

peqGOLD Fastfilter Plasmid Maxiprep Protocol

Supplied by user:

! 100 % Ethanol
! Sterile, deionized water (optional)
! Sterile pipette tips
! Sterile centrifuge tubes (50 ml) – e.g. Falcon® Tubes

1. Bacterial Culture

Inoculate 200-500 ml LB/ampicillin (50 µg/ml) medium placed in a 1-4 liter culture flasks with *E.coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 h. For best results use a overnight culture (~1 ml) as the inoculum. It is strongly recommended that an *endA* negative strain of *E.coli* be used for routine plasmid isolation. Examples of such strains include DH5"® and JM109®.
Pellet up to 200 ml bacteria in appropriate vessels by centrifugation at 3,500 - 5,000 x g for 10 min at room temperature. A 250 ml centrifuge bottle is recommended.
Decant or aspirate medium and discard. To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the walls of the vessel.

2. Lysis of bacteria

To the bacterial pellet add 10.0 ml Solution I / RNase A. Resuspend cells completely by vortexing and/or pipetting.
Complete resuspension of cell pellet is vital for obtaining good yields.

Add 10.0 ml Solution II and gently mix by inverting and rotating tube 7-10 times to obtain a cleared lysate. A 3-5 min incubation at room temperature may be necessary.
Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity.

3. Neutralisation of Lysate

Prepare a Lysate Clearance Filter Syringe by removing the plunger and place the barrel in a tube rack to keep the syringe upright.

Add 10 ml Neutralization Buffer and gently mix by inverting several times until a flocculent white precipitate forms. This may require a 2-3 min incubation at room temperature with occasional mixing.

Add 10ml of GBT Buffer and gently mix by invert 1-2 times. Immediately pour the lysate into the barrel of the Lysate Clarence Filter Syringe. Allow the cell lysate to sit for 5 minutes. The white precipitate should float to the top.

The cell lysate may start to pass through the filter, use a new 50 ml tube to collect the cell lysate. Insert the plunger back into the barrel of the syringe.

Hold the filter syringe barrel over the 50 ml tube and gently insert the plunger to expel the cleared lysate to the tube.

Note: Some of the lysate may remain in the flocculent precipitate, do not force this residual lysate through the filter.

4. Load and Bind

Equilibrate the DNA maxi column: Take a HiBind® DNA Maxi Column pre-inserted in a 50 ml centrifuge tube and add 10 ml Buffer GPS into the HiBind® DNA Maxi column. Centrifuge at 3000 x g for 2 minutes. Discard the flow-through and re-use the collection tube.

Add 20 ml of cleared lysate into the DNA maxi column, centrifuge at 3000 x g for 2 minutes. Discard the flow-through and re-use the collection tube.

Load the remaining of the cleared cell lysate into the DNA maxi column and repeat the spin. Discard the flow-through and re-use the collection tube.

5. Wash I

Add 10ml Buffer HB to the DNA maxi column. Centrifuge at 3000 x g for 2 minutes. Discard the flow-through and re-use the collection tube.

6. Wash II

Add 15 ml DNA wash Buffer to the DNA maxi column and centrifuge at 3000 x g for 2 minutes. Discard the flow-through and re-use the collection tube.

7. Wash III

Add 10 ml DNA wash Buffer to the DNA maxi column and centrifuge at 5000 x g for 10 minutes. Discard the flow-through and collection tube.

8. Elution

Transfer the DNA maxi column to a new 50 ml collection tube (provided). Add 3 ml DNA Elution Buffer or water to the column. Centrifuge at 5000 x g for 3 minutes to elute the DNA.

Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

DNA concentration = Absorbance260 x 50 x (Dilution Factor) µg/ml

High copy number plasmids generally yield up to 1 mg of DNA from 500 ml culture. The ratio of (Abs260)/(Abs280) gives an indication of nucleic acid purity. A value greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatamers may also be present.

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Ordering Information

For Isolation of Plasmid DNA

Product	Ord.-No.	Size
peqGOLD Fastfilter Plasmid Midi Kit	12-6905-01 12-6905-02 12-6905-03	10 Preparations 50 Preparations 200 Preparations
peqGOLD Fastfilter Plasmid Maxi Kit	12-6924-01 12-6924-02 12-6924-03	5 Preparations 20 Preparations 40 Preparations
peqGOLD Plasmid Miniprep Kit I (Safety-Line)	12-6943-00 12-6943-01 12-6943-02	5 Preparations 50 Preparations 200 Preparations
peqGOLD Plasmid Miniprep Kit I (Classic-Line)	12-6942-00 12-6942-01 12-6942-02	5 Preparations 50 Preparations 200 Preparations
peqGOLD Plasmid Miniprep Kit II (Safety-Line)	12-6946-00 12-6946-01 12-6946-02	5 Preparations 50 Preparations 200 Preparations
peqGOLD Plasmid Miniprep Kit II (Classic-Line)	12-6945-00 12-6945-01 12-6945-02	5 Preparations 50 Preparations 200 Preparations
peqGOLD Endo-free Plasmid Midi Kit	12-6915-01 12-6915-02	10 Preparations 50 Preparations
peqGOLD Endo-free Plasmid Maxi Kit	12-6926-01 12-6926-02	6 Preparations 24 Preparations

Troubleshooting Tips

Problem	Likely Cause	Suggestion
Low DNA yields	Poor cell lysis	• Only use LB or YT medium containing ampicillin. Do not use more than 500ml. • Cells may not be dispersed adequately prior addition of Solution II • Solution II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, / 1 % SDS
	Bacterial culture overgrown or not fresh	• Do not incubate culture more than 16h at 37°C
No DNA eluted	DNA wash buffer concentrate not diluted with EtOH	• Prepare wash buffer as discribed.
High molecular weight DNA contamination of product	Over mixing of cell lysate upon addition of solution II	• Do not vortex or mix aggressively after adding solution II
Optical densities do not agree with yield on agarose gel	Trace contaminants eluted from column increase A260	• Make sure to wash column as instructed.
RNA visible on agarose gel	RNase A not added to solution I.	• Add vial of RNase A to solution I.
Plasmid DNA floats out of wells of gel	EtOH trace not completely removed	• Centrifuge column at 3,000 x g for 10 minutes to dry the column or perform a EtOH precipitation.