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BIO BASIC INC.

EZ-10 Spin Column Endotoxin-Free Plasmid Preps Kit

BS71918

Version 4.0
ISO9001 Certified

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EZ-10 Spin Column Endotoxin-Free Plasmid Preps Kit

BS71918:

Components	**BS71918 50 Preps**
Solution I	6 ml
Solution II	12 ml
Solution III	25 ml
Rnase A solution(10mg/ml)	120 ul
Wash solution	20ml
Elution Buffer	5 ml
Liquid Endotoxin Eliminator	5 ml
EZ-10 spin Column & Collection Tube	50
Protocol	1

Storage

Transportation at ambient temperature. Store kits components at 4°C. The kit is stable for one year.

Introduction

The kit provides a simple, rapid and efficient method for plasmid DNA isolation from bacteria using a rapid spin-column. DNA in lysate is selectively absorbed on the column while other impurities such as proteins and salts are eliminated. Average yield of DNA is around 10 µg. The kit also produces Endotoxin-Free plasmid by combining alkali lysis methodology with a unique Endotoxin Eliminator reagent. Final DNA is ready for further research application such as transfection, expression in animal cell strains, or as nucleotide vaccine.

Feature

- ü Fast.
- ü High purity with OD260/OD280 ratio of 1.8-2.0
- ü Versatile and suitable for low and high copy plasmid
- ü Competitive Price
- ü Minimum Endotoxin contaminations

Notice:

1. Before use, add the RNase A Solution to the bottle containing Solution I and mix well. Solution I with RNase should be stored at 4ºC for frequent use and at -20ºC for infrequent use.

2. Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37ºC.

3. Before use, add 48ml of 96-100% of ethanol to 12ml Wash Solution.

4. If Liquid Endotoxin Eliminator becomes layered or turbid, please keep on ice for 10min and then mixed thoroughly.

Procedures

1. Add 1.5ml overnight culture to a 1.5ml microfuge tube and centrifuge at 12,000rpm for 1 minute. Drain the liquid completely.

2. Add 100ul of Solution I into the pellet, mix gently. Keep at room temperature for 2 minutes.

3. Add 200ul of Solution II to the mixture, mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute.

Notes*: To prevent contamination from genomic DNA, do not vortex. Whole lysis time should not exceed 5 minutes.*

4. Add 350ul of Solution III, and mix gently. Incubate at room temperature for 2 minutes.

5. Centrifuge at 12,000rpm for 10 minutes.

6. Transfer above supernatant (step 5) into EZ-10 Spin Column. Centrifuge at 12,000rpm for 1 minutes.

7. Discard the flow-through in the tube. Add 500ul of Wash solution to the column, and centrifuge at 10,000rpm for 1 minute.

8. Repeat wash procedure in step 7.

9. Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional 2 minutes to remove any residual Wash solution.

Note: *To increase elution efficiency, keep EZ-10 spin column at room temperature for 10 minutes or in dryer at 50 ºC for 5 minutes to help evaporating residual ethanol completely.*

10. Transfer the column to a clean 1.5ml microfuge tube. Add 50ul of Elution Buffer into the center part of the column. Let the column sit at room temperature for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes.

Note: *Incubating the column with the Elution Buffer at higher temperature (37ºC to 50ºC) may slightly increase the yield especially of large DNA Plasmids. Prewarming the Elution Buffer at 55ºC to 60ºC may also slightly increase elution efficiency.*

11. Add ddH_2O or TE buffer into plasmid sample elution solution so that final volume is 250ul. Then add 25ul 3M acetate sodium (pH 5.2) solution and mixed thoroughly, keep at ice for five minutes.

12. Add Pre-cold 25ul Liquid Endotoxin Eliminator. Then mix thoroughly and keep on ice for 10 minutes.

13. Heat mixture in water bath at 65 ºC until the solution become turbid or layered, usually this takes 1-5 minutes.

14. Centrifuge at 12000rpm for 2 minutes at >25 ºC.

Note: *In order to have a visible interface between top layer and bottom layer, one must centrifuge at the right temperature > 25 ºC. Otherwise there is no two-phase layer formation if operate at low temperature.*

15. Transfer top layer phase into a new Endotoxin-free 1.5ml centrifugation tube with Endotoxin-free tip.

16. Optional: Repeat step 12-15 for three times to further reduce endotoxin level.

17. Add 3 volumes of COLD ethanol into above mixture from step 16. Mix and then keep on ice for 30 minutes.

18. Centrifuge at 12000rpm for 10 minutes and discard supernatant.

19. Wash the precipitation with 75% COLD ethanol twice. Spin at 12000rpm for 5 min and discard supernatant.

19. Dissolve the plasmid in Endotoxin-free water. Such water is treated by Millipore or Hyclone ultrapure system. Alternatively, one can directly dissolve sterile water for injection.

20. Keep DNA at -20 ºC till further use.