DANAGENE SPIN MINIPREP KIT

REF.0702.1 250 MINIPREPS
REF.0702.2 1000 MINIPREPS

1.INTRODUCTION

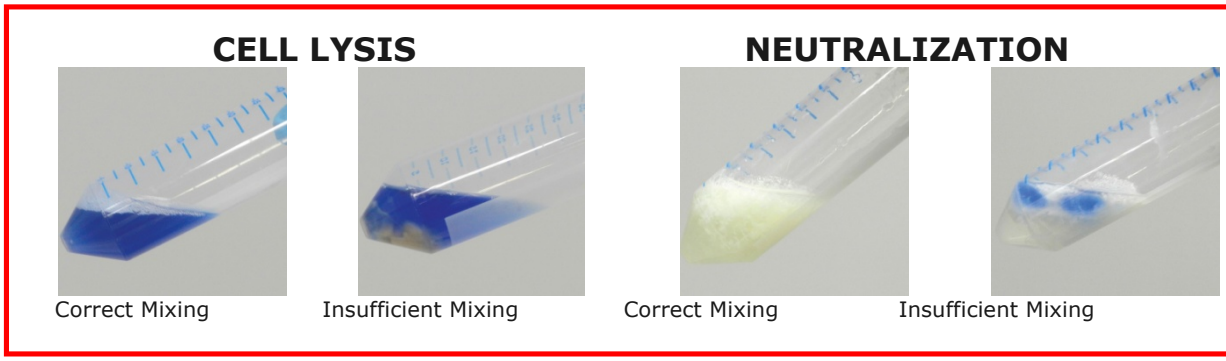
This kit is designed for the rapid , small scale preparation of high purity plasmid DNA from E. coli cultures of 1.5-3.0 ml. It´s possible to prepare around 24 minipreps in less than 45 minutes obtaining a high purity plasmid DNA.

The method uses an improved alkaline lysis method, where organixc toxic reagents are not used, using instead chaotropic salts which promote the selective DNA binding to a **glass fiber matrix prepared in MicroSpin columns.** The plasmid DNA is purifed with several washing steps to remove impurities and finally eluted.

The DNA obtained can be used in different applications, includding DNA automated sequencing by capilar electrophoreis**.**

It introduces **TRUEBLUE Lysis control reagent** a color indicator wich provides visual identification of optimum buffer mixing. This prevent common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA and cell debris. This makes ideal for use by researchers who have not much experience with plasmid preparation as well as experienced scientists who want to be assured of maximum product yield.

Visualization of efficient cell lysis and SDS precipitation using TRUEBLUE Lysis control reagent



Features:

- **Purify plasmid DNA within 15 minutes.**
- **Convenient:** Plasmid silica fiber spin column
- **Plasmid Size:** 1-15 kb.
- **High Yield:** up to 20 µg of pure plasmid DNA.
- **Sample Volume:** 1.5-3.0 ml of cultured bacterial cells.

2. KIT COMPONENTS

	Ref. 0702.1 250 preps	Tª
Resuspension Solution	65 ml	Room temperature
Lysis Solution	65 ml	Room temperature
Neutralization/Binding Solution	90 ml	Room temperature
Wash Solution	70 ml	Room temperature
Elution Buffer	30 ml	Room temperature
RNase	10 mg	4ºC
TRUEBLUE Lysis control reagent	750 µl	Room temperature
Spin Columns	250 unid.	Room temperature
Collection Tubes	250 unid.	Room temperature

Equipment and additional reagents required

* 100 % Ethanol
* 1.5 ml microtubes
* Microcentrifuge.

3. GENERAL PROTOCOL

3.1 Preliminary conditions

- Several factors can interfere in the plasmid DNA obtaining. These includes the number of copies of the vector, the DNA insert, the strain host, the medium and growing conditions
- The use of host strains that contain a mutation in the Endonuclease I (end A) gene are commendable. Such as JM109, DH5alpha, DH10B, XL1-Blue. DNA extraction from strains that contain the product of the endonuclease I gene, such as HB101 and MC106, can produce samples with traces of endonucleases, for this reason, these strains should be avoided. If it is necessary to use these strains contact **DANAGENE** Laboratory Technical Service and we´ll supply you with an additional protocol.

3.2 Preliminary Preparations

- **ATENTION:** The Neutralization/Binding Solution contains guanidine hydrochloride that is an irritant agent, it is recommeded to use gloves and glasses.
- The Resuspension solutions already contain RNase. The kit includes an extra vial with RNase for that customers who need and extra quantity of this enzyme in order to obtain an RNA-free plasmídic DNA. If you need it, dissolved the RNase powder into the Resuspension Solution and then store it at **4ºC,** this prepared solution is stable during 6 months
- Verify that the Lysis Solution does not contains precipitated SDS due to the low temperatures. If necessary, dissolve the SDS heating at 37ºC.
- **Add 280 ml of Ethanol 100 % to the Wash Solution** . Keep the container closed to avoid the evaporation of the ethanol.
- The elution can be done at room temperature although the yield can be increased a 20 % if the Elution Buffer is heated at 55ºC.

3.3 Protocol for plasmid DNA extraction from 1.5-3.0 ml cultures

1. Centrifuge **1.5 ml** of an overnight culture of E.coli at > 12.000 x g for 60 seconds. Remove the supernatant by aspiration without disturbing the pellet. You can repeat harvesting of 1.5 ml culture using the same microcentrifuge tube.

NOTE: Using OD600=2-6 units of bacterial culture is recommended. Do not use overgrown bacterial cultures (< 16 hours incubated with mixing at 37ºC). Use a fresh bacterial cultures only.

2. **Resuspend the pellet in 250 µl of Resuspension Solution EP01+ 2.50 µl de TRUEBLUE Lysis control reagent by vigorous vortexing**, assure the complete cells resuspension.

NOTE: Make sufficient Resuspension solution / TRUEBLUE Lysis control reagent working solution for the number of plasmid preps being performed. TRUEBLUE precipitates after addition to resupension solution, this precipitate will completely dissolve after addition lysis solution. Shake before use to resuspend the TRUEBLUE particles.

3. **Lyse the cells with 250 µl of Lysis Solution.** Carefully invert the tube 8-10 times until de mixture appears clear and viscose. Do not use vortex. It can be incubated for 1-3 minutes, but never for more than 3 minutes.

4. **Neutralize with the addition of 350 µl of Neuralization/Binding Solution.** Carefully invert the tube 8-10 times. It is recommended to incubate in ice for 5 minutes.

5. **Centrifuge at maximum speed in a microcentrifuge for 5minutes.** If there were floating particles in the supernatant, centrifuge once again.

6. **Carefully pour the supernatant to a MicroSpin column with its collection tube.**

7. **Centrifuge at maximum speed for 30-60 seconds.** Take the MicroSpin column out from the collection tube, remove the liquid and place the spin microcolumn back in the same collection tube.

8. **Add 600 µl of Wash Solution to the MicroSpin column. Centrifuge at maximum speed for 30-60 seconds.** Remove the liquid.

9. **Make 2nd wash. Add 600 µl of Wash Solution to the MicroSpin column. Centrifuge at maximum speed for 30-60 seconds.** Remove the liquid.

10. **Centrifuge at maximum speed for 90 seconds to remove the residual ethanol.**

11. **Insert the MicroSpin column in a new 1.5 ml tube. Add 50-100 µl of Elution Buffer to the spin microlcolumn.**

12. **Incubate 1 minute.** It is recommended that the buffer is at 55ºC.

13. Centrifuge at maximum speed for 60 seconds.

4. TROUBLESHOOTING

1. ***Low or negative DNA obtaining:***
   1.1. ***Due to an insufficient number of cells*.:** *Possibly the culture is old. Prepare a new culture. Confirm the cell density.*
   1.2. ***Due to a poor plasmid replication***: *Confirm that the cells were grown in a suitable medium under optimum conditions.*
   1.3. ***Due to an insufficient antibiotic activity***: *Many antibiotics are light sensible and get degraded when it is stored for a long time at 2-8 ºC.*
   1.4. ***Due to a long alkaline lysis*:** Reduce the lysis time.
   1.5. ***Due to an incomplete cell remains precipitation***: *Reduce the culture initial volume.*
   1.6. ***Due to an incomplete lysis****: Reduce the culture initial volume or increase the lysis time.*

2. **Low quality or contaminated DNA:**
   2.1. ***Genomic DNA****: The bacteria lysate mixture has been done too strongly. Do not use vortex. Do not use cultures that have grown more than 24 h.*
   2.2. ***Genomic and degraded DNA:*** *Try to avoid that any particle passes from the lysate to the supernatant collected in point 7 of the protocol.*
   2.3. ***ARN*.**: *The digestion with RNAse was not enough. Check that the RNAse has been added to the Resuspension Solution. If this solution is more than 6 months old, it is recommended to add more RNAse.*
   2.4. ***Nucleases***: *Check that the solution does not have nucleases.*

3. **Low ratio $A_{260/280}$ in the purified DNA:**
   3.1. ***The purification is incomplete due to a large DNA quantity****: Reduce the cell culture initial volume.*
4. **Difficulty to redissolve the plasmidic DNA:**
   4.1. ***The pellet was over dried:*** *Dry it and heat the TE or sterile water at 60-70ºC.*
   4.2. ***Low pH***: *Check theTE pH is >8.0.*
   4.3. ***Resuspending volume too low.***

5. **Additional plasmid bands.**
   5.1. ***The denatured plasmid DNA appears as a band below the super helicoidal DNA****. The lysis reaction must not be more than 3 minutes long.*

We recommend contact with ***DANAGEN*** *laboratory technical service for any additional question regarding the work protocols or any problems you may have during work*.