Plasmid DNA Miniprep Kit

Description

Materials Provided:

Material	100 Prep	5 Prep
***Resuspension**Â **Buffer**	30 ml	1.5 ml (RNase
Rnase A Solution (10 Âµg/Âµl)	300 Âµl	â€“
Lysis Buffer	30 ml	1.5 ml
Neutralization Buffer	30 ml	1.5 ml
DNA Binding Buffer (Brown Bottle)	40 ml	2 ml
****Wash Buffer**	20 ml (addÂ **80 mlÂ** of 96 -100 % ethanol	1 ml (addÂ **4 m** 100 % ethano
Elution Buffer	20 ml	1 ml
Mini Columns	100	5
Tubes as column inserts	100	5

Additional requirements:Â Â Â Â Microcentrifige Tubes , 96 â€“ 100% Absolute ethanol.

**Note: ****To Wash Buffer add 96-100% ethanol (In 20 ml of wash buffer + 80 ml ethanol).

***Add Rnase A Solution 300 Âµl to 30 ml Resuspension Buffer.**

Procedure:

1. Pipette about 1 ml of coli cells into a 1.5 ml microfuge/Eppendorf tubes. Centrifuge the sample at 10,000 rpm for 2-5 minutes at room temperature.
2. Discard the supernatant, and resuspend the cell pellet in 250 Âµl of Resuspension Buffer containingÂ **RNase AÂ** . Mix by tapping
3. Add 250 Âµl of Lysis Buffer to the cellÂ **(Do not vortex)**
4. Mix the suspension by gently tapping or by inverting the tube up and down 8-10
5. Add 250 Âµl of Neutralization Buffer and mix the solution thoroughly by inverting the tube up and down 8-10Â **(Do not vortex).**
6. Centrifuge at 10,000-14,000 rpm for 10 Discard the pellet and save the supernatant.
7. Add 375 Âµl of DNABinding Buffer to the clear supernatant and
8. Load 550-600 Âµl of the mixture on to the DNA spin column, centrifuge for 1 -2 minutes and discard the flow

Note: You can save the remaining half of the lysate and freeze it at -20Â°C for

future use. If you plan to use all of it now, this will probably double the amount of the DNA yield.

9. Wash the DNA spin column with 400 Âµl of Wash Centrifuge the column for 1-2 minutes. Discard the flow through. Wash one more time.

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