For life science research only.
Not for use in diagnostic procedures.

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BehMag Plasmid Extraction Kit

Small-Scale Preparations of DNA Isolation based on Magnetic beads

Components

Catalog No.	25 preps	50 preps
LB Buffer	5ml	10ml
Magnetic Beads	1.5.ml	3 ml
RNase A	15 ul	30 ul
SB Buffer	8ml	15ml
NB buffer	10ml	20ml
WB Buffer	15ml	30ml

EB Buffer	5ml	10ml

Stored at room temperature

Expiration: 1 year

Companion Device----------

Magnetic separation rack

Equipment & Reagents to be supplied by user----------

- Pipets and pipet tips
- 1.5 ml Microtube
- Vortex
- Dry Heat Block/ Water Bath
- Magnetic bead separation rack
- Micropipettors
- 70% ethanol (prepare fresh)
- 96-100% Isopropyl
- Nuclease-free water

*The Buffer TN contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Procedure

Notes prior to use:

• Ensure that all solutions, except the Resuspension Solution SB, are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.

• Add the entire amount of RNase A to the Resuspension Solution SB. The label on the bottle has a box that can be checked to indicate that the RNase has been added. The solution can be stored for up to 6 months at 4°C.

• Bacterial cultures grown overnight at 37oC in LB medium are optimal for this procedure.

• Preheat an incubator to 65°C

Precautions and Disclaimers----------

- This kit has been designed for research purposes only. It is not intended for human or diagnostic use.
- Avoid freeze/thaw cycles and centrifugation which could damage the beads.
- Be sure to vortex magnetic beads before using
- Vortex samples 10 seconds before adding

Description----------

Designation is here made of BehMag plasmid Extraction kits in order to quickly prepare plasmid DNA from bacterial cultures. Magnetic NPs are designed to be able to best bind DNA where might well have optimized concentrations of salts, whereas they are intended to release the said plasmid DNA under alkali and low-salt conditions. Preferably, the kit is isolated from other cellular elements such as genomic DNA and RNA. Nearly all of tested restriction enzymes are well capable of digesting the obtained plasmid DNA. In addition, eliminating impurities could ultimately boost the performance in a variety of different applications. It should be noted that further centrifugation or isopropanol precipitation are not required during the purification process.

Specifications

- High-speed: fast purification of plasmid from cell culture (30 min)
- High yield: 50µl of magnetic beads can bind 30µg plasmid DNA
- High recovery: recovery of DNA fragments is up to 95% (Up to 20 µg from 1.5 mL)
- Size of plasmids purified : Up to 13,000 bp
- Fast, reproducible and easy processing using a Magnetic bead system
- Isolate high quality plasmid DNA
- Recovered plasmid DNA is compatible with various downstream applications of Culture)
- Automation: no liquid-liquid mixing and separation process; therefore, the whole procedure can be easily automated

Protocol

1. Lysate Preparation

a. Transfer 1.5 mL of bacterial culture to a microtube and centrifuge for 30 seconds to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

b. Add 200 µL of Resuspension Solution SB (containing RNase A; see Notes Prior to Use) to the cell pellet. Resuspend the cells by pipetting in and out, or by gentle vortexing. Incubate at room temperature for 5 minutes.

c. Add 250 µL of LB to the cell suspension, cap the tube, and mix the contents by gently inverting the tube several times. Do not vortex as this will shear the genomic DNA. The suspension should become clear and viscous as the cells begin to lyse. Continue mixing until the mixture becomes clear. If necessary, allow the solution to incubate at room temperature provided the total incubation time is no more than 5 minutes. This step is also critical for the denaturation of cellular proteins and genomic DNA.

d. Add 350 µL of Buffer NB and immediately mix by inverting the tube several times. The solution will become turbid as insoluble particles from denatured materials start to form.

e. Centrifuge for 10 minutes to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.

f. Transfer the lysate into a fresh 1.5 mL microtube.

g. Add 20 µL of Magnetic Bead Suspension (vortex prior to use) to the mixture above.

h. Incubate at room temperature for 5 minutes. Occasionally invert the tube.

i. Proceed to Section 2: Plasmid DNA Isolation.

2. Plasmid DNA isolation

a. Place the sample tube in the magnetic rack. Allow to sit for 30 sec.

b. Aspirate and discard supernatant without touching the magnetic beads.

c. Remove the sample tube from the magnetic rack and gently add 300 ul WB buffer Resuspend by vortexing or pipetting for30 sec

d. Place the sample tube on the magnetic rack and allow to sit for 30 sec.

e. Aspirate and discard supernatant without touching the magnetic beads.

f. Repeat Steps 2c – 2e for a second wash step. Note: Remove as much of the WB buffer in the sample tube as possible by pipetting.

g. Incubate the open tube at 60˚C for 5 minutes to dry the magnetic beads.

h. Remove the sample tube from the magnetic rack and add 50 µL of EB buffer. Mix by vortexing and incubate at 60˚C for 15 minutes.

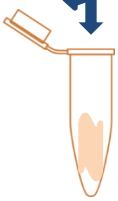
i. Briefly spin for 10 sec and place sample tube on the magnetic rack.

j. Carefully transfer the elution to a fresh 1.5 mL microtube without touching the magnetic beads. The purified plasmid DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at –20°C for long-term storage

Flow Chart

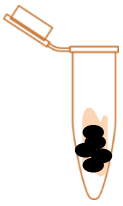
Overnight cultures

Spin



Adding SB buffer
Adding LB buffer
Adding NB buffer

Spin



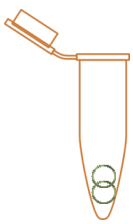
Adding Magnetic beads

Applying Magnetic field
Discarding the supernatant

Washing the beads

Adding EB buffer

Incubate at 55°C for 10 min



Transferring supernatant to a clean tube.