*B*IOMEDICAL *R*ESEARCH *S*ERVICE *C*ENTER
*U*NIVERSITY *at B*UFFALO

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Mini-Plasmid Isolation Kit (Cat #: N-102)

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COMPONENTS: Plasmid Solution-1 (PS-1): 30 ml, store at 4ºC (sufficient for 300 mini-preps)
Plasmid Solution-2 (PS-2): 30 ml, **store at room temperature**
Plasmid Solution-3 (PS-3): 30 ml, store at 4ºC
Plasmid Solution-4 (PS-4): 5 ml, store at 4ºC

PRODUCT DESCRIPTION: Plasmid DNA can be rapidly purified from an overnight E. coli culture in 15 min with a typical yield of 1-5 μg DNA (as determined by plasmid replication origin) per ml of culture without additional RNase treatment and phenol-chloroform extraction steps. The DNA is suitable for restriction digestion, probe preparation, and PCR analysis. DNA to be used for sequencing analysis is subjected to an additional purification step. The kit is stable for at least one year if handled and stored properly. The product is for research use only.

REGULAR PROTOCOL:

1. Transfer 1-1.5 ml of overnight shaking bacterial culture to 1.5-ml microtubes, and deposit cells by a 20-sec spin in a microfuge. Aspirate off medium completely. Carry out the following steps at room temperature.
2. Add 0.1 ml PS-1 to each tube, and vortex vigorously to resuspend cells.
3. Add 0.1 ml PS-2 to each tube, close tubes, gently invert tubes ten times, and wait 30 sec.
4. Add 0.1 ml PS-3 to each tube, close tubes, mix contents by brief vigorous vortexing, and wait 5 min.
5. Spin tubes 2 min. Transfer supernatant to another set of 1.5-ml microtubes (AVOID CARRYING OVER FLOATING DEBRIS). Precipitate DNA by adding 0.15 ml isopropanol followed by mixing. Wait 5 min. Note that adding too much isopropanol or cold temperature can increase RNA contents in your mini-preps.
6. Spin tubes 5 min. Aspirate off supernatant completely. Vortex pellet in 0.3 ml 70% ethanol for 10 sec followed by a 2-min spin. Carefully aspirate off ethanol completely. Air dry pellet. Bring up DNA in 30-50 μl of sterile water by vortexing. The DNA is now ready for restriction digestion and PCR analysis.

PROTOCOL FOR SEQUENCING-GRADE PLASMID DNA:

1. Deposit ~3 ml of overnight shaking bacterial culture to 1.5-ml microtubes by spinning twice (see above). Aspirate off medium completely.
2. Repeat steps 2-6 above using 0.2 ml (not 0.1 ml) of each Plasmid Solution. Bring up DNA in 50 μl sterile water by vortexing.
3. Spin tubes 5 min to deposit residual debris (important step). Transfer the clarified DNA solution to a set of 0.5-ml microtubes. Precipitate DNA by mixing with 25 μl PS-4. Keep tubes on ice for 10 min.
4. Spin tubes 5 min, aspirate off supernatant, and vigorously vortex pellet (invisible at this point) in 0.3 ml 70% ethanol for 30 sec. Spin tubes 2 min, and aspirate off ethanol completely. Air dry pellet. Bring up DNA in 50 μl of sterile water by vortexing.
5. Measure DNA concentration by absorption at 260 nm. Dilute DNA with sterile water to ~250 ng/μl. The plasmid DNA is now ready for DNA sequencing.

NOTE:
- Exercise caution in handling PS-2 solution, which contains high concentrations of SDS and NaOH (see website MSDS). Use sterilized tips and tubes for all steps.
- Avoid using E. coli strains HB101 and TG1 for plasmid isolation.
- Isopropanol and 70% ethanol are required for plasmid isolation, but are not included in the kit.