

Plasmid DNA isolation:

(Plasmid pET-15b-irisin is isolated from the E. coli DH5-alpha strain)

1. Pellet 1–5 ml (not to exceed 15 OD units) bacterial culture by centrifugation for 30 seconds. Discard supernatant.
1.5 ml of culture is sufficient for most applications. Ensure cultures are not overgrown (12-16 hours is ideal).
2. Resuspend pellet in 200 µl Plasmid Resuspension Buffer (B1) . Vortex or pipet to ensure cells are completely resuspended. There should be no visible clumps.
3. Add 200 µl Plasmid Lysis Buffer (B2) , gently invert tube 5–6 times, and incubate at room temperature for 1 minute. Color should change to dark pink, and solution will become transparent and viscous. Do not vortex.
4. Add 400 µl of Plasmid Neutralization Buffer (B3) , gently invert tube until neutralized, and incubate at room temperature for 2 minutes. Sample is neutralized when color is uniformly yellow and precipitate forms.
Do not vortex.
5. Centrifuge lysate for 2–5 minutes. For best results, and especially for culture volumes > 1 ml, we recommend a 5 minute spin to ensure efficient RNA removal by RNase A. Pellet should be compact; spin longer if needed.
6. Carefully transfer supernatant to the spin column and centrifuge for 1 minute. Discard flow-through.
7. Re-insert column in the collection tube and add 200 µl of Plasmid Wash Buffer 1. Centrifuge for 1 minute. Discarding the flow-through is optional.
8. Add 400 µl of Plasmid Wash Buffer 2 and centrifuge for 1 minute.
9. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow-through. If there is any doubt, re-spin the column for 1 minute.
10. Add ≥ 30 µl DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA. Nuclease-free water (pH 7-8.5) can also be used to elute the DNA. Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated. For larger size DNA, (≥ 10 kb), heating the elution buffer to 50°C prior to use can improve yield.