User-Developed Protocol:

Isolation of genomic and plasmid DNA from cell cultures using the QIAGEN® Genomic-tip

This procedure has been adapted by customers from the QIAGEN® Sample Preparation and Lysis Protocol, and is for use with the QIAGEN-tip or QIAGEN Genomic-tip. **It has not been thoroughly tested or optimized by QIAGEN.**

Please be sure to read the *QIAGEN Genomic DNA Handbook* and the detailed Cell Cultures Protocol carefully before beginning this procedure.

Important note before starting

- The maximum binding capacity for QIAGEN Genomic-tip 20/G (mini) is 20 µg, QIAGEN Genomic-tip 100/G (midi) is 100 µg, and QIAGEN Genomic-tip 500/G is 500 µg. Choose the appropriate QIAGEN Genomic-tip for the expected plasmid yield, regardless of starting material and corresponding lysis buffer volumes.

Procedure

1. **For 0.5 ml, 2 ml, or 10 ml cell suspension (1 x 10^7 cells/ml), add 1 volume (0.5, 2, or 10 ml) of ice-cold Buffer C1 (4x concentrate) and 3 volumes of ice-cold water (1.5 ml, 6 ml, or 30 ml). Mix by inverting the tube several times. Incubate for 10 min on ice.**
2. **Centrifuge the lysed cells at 1300 x *g* for 15 min at 4°C to pellet the nuclei. Save the supernatant – it contains the plasmid DNA. To isolate genomic DNA, use the pellet and continue with Step 5 of the Sample Preparation and Lysis Protocol for Cell Cultures in the *QIAGEN Genomic DNA Handbook*.**
3. **Incubate the supernatant with 100 µg/ml RNase A for 30 min at 37°C.**
4. **Add 750 mM NaCl to the supernatant to adjust the binding conditions.**
5. **Equilibrate the appropriate QIAGEN-tip with Buffer QBT (2 x 1 ml for Mini, 4 ml for Midi, or 10 ml for Maxi).**
6. **Apply supernatant to the column and allow it to enter by gravity flow.**
7. **Wash with 4 x 1 ml, 2 x 10 ml, or 2 x 30 ml of Buffer QC.**
8. **Elute DNA with 0.8 ml, 5 ml, or 15 ml of Buffer QF.**
9. **Precipitate with 0.7 volumes of isopropanol previously equilibrated to room temperature. Centrifuge immediately at 15,000 x *g* for 30 min at 4°C. Carefully discard the supernatant.**
10. **Wash DNA with 1 ml, 5 ml, or 15 ml of 70% ethanol. Air-dry for 5 min and dissolve the pellet in a suitable volume of buffer.**

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected handbooks can be downloaded from **www.qiagen.com/literature/handbooks/default.asp**.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp**.