User-Developed Protocol:

Isolation of genomic and viral DNA from lymphocytes using the QIAGEN® Genomic-tip

This procedure has been adapted by customers from the Genomic-tip Protocol, and is for use with the 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.

Procedure

1. **Centrifuge lymphocytes to form a pellet. Discard the supernatant.**
2. **Resuspend pellet in Buffer C1 as described in the Sample Preparation and Lysis Protocol for Cell Cultures in the *QIAGEN Genomic DNA Handbook*.**
3. **Centrifuge the lysed cells at 4°C for 15 min at 1300 x *g*. Save the nuclear pellet and the supernatant (S1).**

Viral DNA in cytosolic fraction

3a. **To supernatant S1, add RNase A to a final concentration of 10–20 µg/ml. Add 25 µl QIAGEN Protease or Proteinase K per ml of supernatant.**

4a. **Incubate for 1 h at 50°C.**

5a. **Add NaCl to a final concentration of 750 mM and adjust the pH to 7.0.**

6a. **Load onto an equilibrated QIAGEN Genomic-tip and follow the wash and elution steps in the Genomic-tip Protocol for Isolation of Genomic DNA from Blood, Cultured Cells, Tissue, Yeast, or Bacteria in the *QIAGEN Genomic DNA Handbook*.**

Note: QIAGEN Genomic-tip 20/G should be used for the viral DNA purification steps. If the expected yield of viral DNA is unknown or less than 1 µg, a carrier, for example glycogen, should be added to the eluate before the isopropanol precipitation.

Viral DNA in nuclear fraction — integrated

3b. **Treat nuclear pellet from Step 2 with Buffers P1, P2, and P3 and centrifuge as described in the QIAGEN Midi and Maxi Kits Protocols in the *QIAGEN Plasmid Purification Handbook*. Save the pellet (which contains the genomic DNA) and the supernatant (S2).**

4b. **Resuspend the pellet in Buffer G2, add Proteinase K or QIAGEN Protease, and incubate according to the instructions in Step 7 of the Sample Preparation and Lysis Protocol for Cell Cultures of the *QIAGEN Genomic DNA Handbook*.**

5b. Precipitate the DNA with 0.7 volumes of isopropanol and resuspend DNA in TE. Add Buffer QBT to a final volume of 1 ml for a Genomic-tip 20/G, 10 ml for a Genomic-tip 100/G, or 25 ml for a Genomic-tip 500/G. Load the sample onto the corresponding equilibrated Genomic-tip and follow the wash and elution steps in the *QIAGEN Genomic DNA Handbook* (Step 3 and Step 4 of the Protocol for Isolation of Genomic DNA from Blood, Cultured Cells, Tissue, Yeast, or Bacteria),

Viral DNA in nuclear fraction — not integrated

3c. Load S2 from Step 3b onto the equilibrated Genomic-tip and follow the wash and elution steps in the *QIAGEN Genomic DNA Handbook*.

Note: QIAGEN Genomic-tip 20/G should be used for the viral DNA purification steps. If the expected yield of viral DNA is unknown or less than 1 µg, a carrier, for example glycogen, should be added to the eluate before the isopropanol precipitation.