Promega

Product Application

DNA Recovery from Cell Culture Media and Buffers using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit

Assess DNA contamination in viral samples using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit.

Kit: Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)

Analysis: qPCR

Sample Type(s): Media or buffers used in viral purification from cell-culture viral expression systems

Input: 300µl of culture media or buffer

Materials Required:

- Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Cat.# AS1330)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat block

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM420, available at: **www.promega.com/protocols**

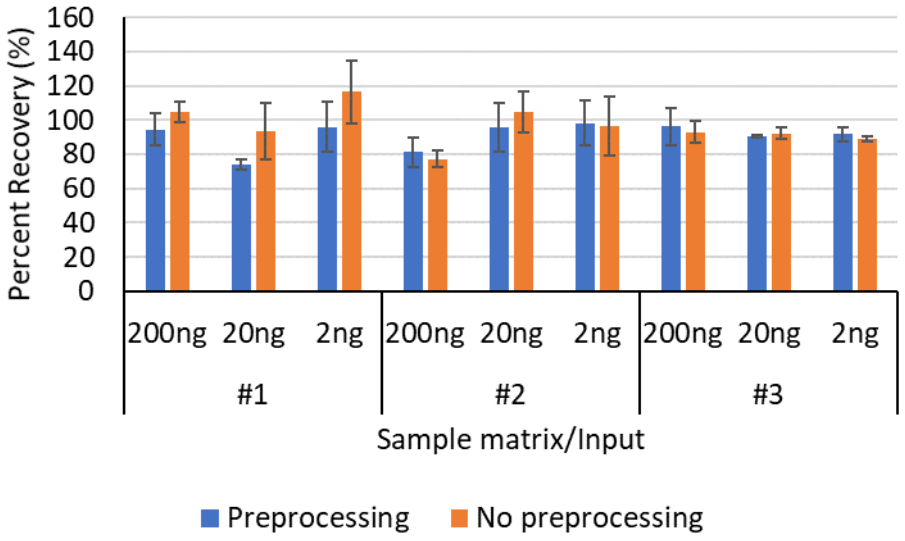
or contact Technical Services at: **techserv@promega.com**

Protocol:

1. If sample lysis is required: transfer 300µl of sample to a clean 1.5ml tube.
    a. If free DNA will be detected, sample lysis may not be required. Skip steps 1-3, add 300µl of sample directly to well #1, and proceed to Step 5.
2. Add 300µl of Lysis Buffer and 30µl of Proteinase K to the sample.
3. Vortex for 10 seconds.
4. Incubate at 56°C for 10 minutes.
5. Prepare Maxwell® cartridges according to the Technical Manual (TM420, Section 4.C.).
    a. For Step 4 in Section 4.C., add 100µl of Nuclease-Free Water for increased DNA recovery.
6. Transfer entire lysate to well #1 of the Maxwell® RSC cartridge.
7. Select the Maxwell® RSC Viral Total Nucleic Acid run method, place the prepared deck tray in the Maxwell® Instrument, and start the method.

Results:

Human genomic DNA spiked at 200ng, 20ng, or 2ng in 300µl of three representative sample matrices was purified with the Maxwell® RSC Viral Total Nucleic Acid Purification Kit using the protocol described above, and the average percent recovery of the spiked gDNA was determined by qPCR.



Recovery of spiked human gDNA using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit. Sample matrix #1 (30% Expi293 Medium (ThermoFisher™, Cat.# A1435101), 70% FreeStyle 293 Medium (ThermoFisher™, Cat.# 12338001)), sample matrix #2 (13mM Tris, 8mM HEPES, 320mM NaCl, 8% Sucrose) and sample matrix #3 (10mM Tris (pH 7.5), 10mM HEPES (pH 7.5), 100mM NaCl, and 10% sucrose) were spiked with ProNex® DNA QC gDNA Standard (50ng/µl) to a final DNA concentration of 667pg/µl. Each spiked sample matrix was serially diluted 1:10 and 1:100. 300µl of each spiked sample matrix dilution (200ng, 20ng, or 2ng of gDNA) was preprocessed with sample lysis or added directly to well #1 according to the method above. gDNA spiked samples were purified in triplicate and eluted in 100µl of Nuclease-Free Water. Eluate concentrations were determined by qPCR using the ProNex® DNA QC Assay (Cat.# NG1004), and the total gDNA yield was calculated based on the recovered eluate volume. The average percent recovery of the spiked gDNA for each sample processed with (blue) or without (orange) sample lysis is shown above. Percent recovery was calculated based on the qPCR results and the eluate volumes. Error bars represent the standard deviation of three purification replicates.