Promega

Product Application

Automated Purification of HPV DNA from Cells Collected in Saline or Phosphate Buffered Saline

Purify HPV DNA from cell specimens collected in saline or phosphate buffered saline (PBS) with the Maxwell® RSC Viral Total Nucleic Acid Kit and the Maxwell® RSC Instrument.

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

Analyses: qPCR

Sample Type(s): Cells collected in saline or PBS

Input: ≤300µl

Materials Required:

- Maxwell® RSC Viral Total Nucleic Acid Kit (Cat.# AS1330)
- Maxwell® RSC Instrument (Cat.# AS4500)
- ClickFit Microtubes
- Heat Block set to 56°C

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**

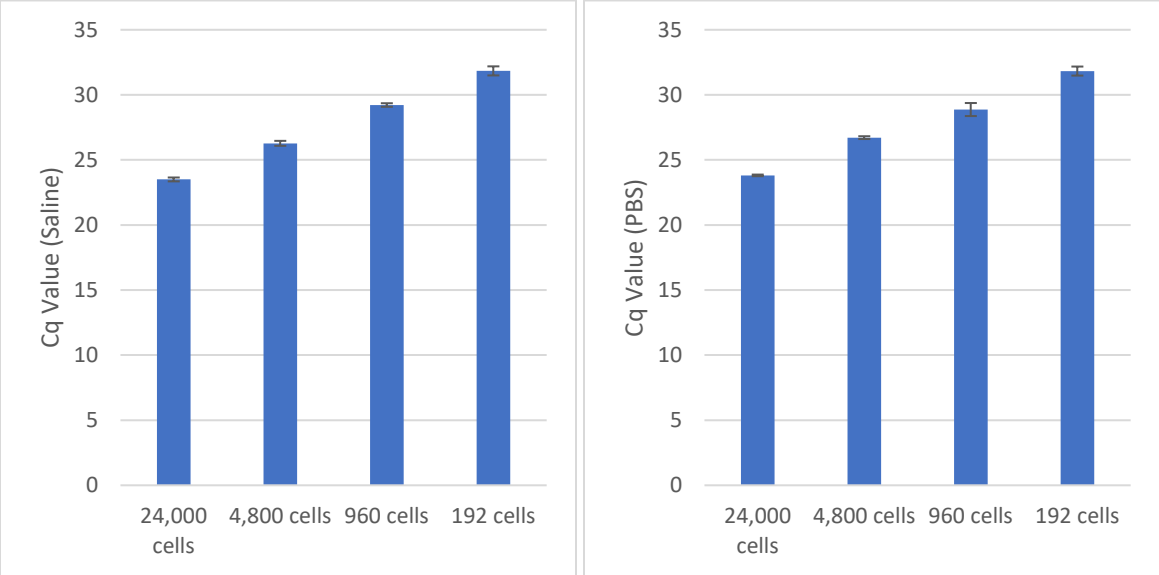
or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Vortex the sample briefly to resuspend cells, and then, working quickly, transfer 300µl of the solution to a 1.5ml ClickFit Microtube.
2. Add 300µl Lysis Buffer and 30µl Proteinase K to each sample. Vortex samples for 10 seconds.
3. Incubate samples at 56°C for 10 minutes.
4. During sample incubation, place the necessary number of cartridges in the Deck Tray, remove the foil seals, and prepare the cartridges as follows.
    a. Place a plunger in well #8 of the cartridge.
    b. Add 50µl of Nuclease-Free Water to the bottom of the elution tubes.
5. Following the sample incubation, add the entire volume of lysate to well #1 of the Maxwell® RSC cartridge.
6. Process samples on the Maxwell® RSC Instrument using the Viral Total Nucleic Acid method.

Results:

HPV-18 DNA was detected in nucleic acid purified from 300µl of saline or PBS spiked with HeLa cells, which are known to be infected with HPV-18.



HPV-18 DNA recovery with the Maxwell® RSC Viral Total Nucleic Acid Kit from 300µl cells collected in saline or PBS. Saline and PBS samples were prepared by spiking the indicated number of HeLa cells into a 300µl sample. 300µl of saline (left panel) or PBS (right panel) containing spiked cells was processed with the Maxwell® RSC Viral Total Nucleic Acid Kit (Cat.# AS1330) as described above. HPV-18 DNA was detected in the eluates using the GoTaq® Probe qPCR Master Mix (Cat.# A6101) with specific primers to HPV-18[1]. Bars represent average Cq value ± standard deviation for triplicate purifications amplified in duplicate.

References:

1. Seaman, W.T., *et al.*, (2010) Detection and quantitation of HPV in genital and oral tissues and fluids by real time PCR, *Virology Journal*. **7**, 194.