

Automated Purification of HPV DNA from Cells Collected in SurePath™ Preservative Fluid

Purify HPV DNA from cell specimens collected in SurePath™ fixative 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 SurePath™ preservative fluid

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.

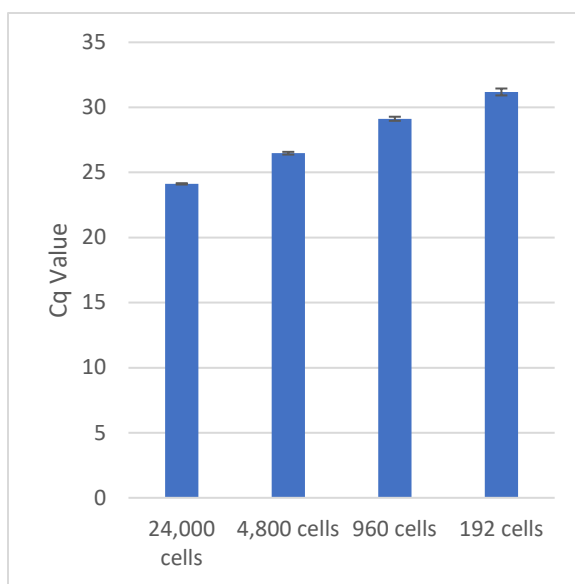
For further information, see Technical Manual TM420, available at:
www.promega.com/protocols
or contact Technical Services at:
techserv@promega.com

Protocol:

1. Vortex the SurePath™ vial 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 vortex samples for 10 seconds.
3. Incubate samples at 80°C for 30 minutes.
4. Remove samples from heat block and allow samples to cool at room temperature for 5 minutes.
5. Add 30µl Proteinase K to each sample, and vortex for 10 seconds.
6. Incubate samples at 56°C for 10 minutes.
7. During final 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.
8. Following the sample incubation, add the entire volume of lysate to well #1 of the Maxwell® RSC cartridge.
9. 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 SurePath™ preservative fluid 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 SurePath™ preservative fluid. SurePath™ preservative fluid was spiked with the indicated number of HeLa cells in a 300µl sample. 300µl of sample 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/probe to HPV-18¹. 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.