Promega

Product Application

Total nucleic acid purification from feces with the ReliaPrep™ Blood gDNA MiniPrep System

Isolation of pure, amplifiable nucleic acid from human feces using the ReliaPrep™ Blood gDNA MiniPrep System.

Kit: ReliaPrep™ Blood gDNA Miniprep System

Analyses: RT-qPCR, qPCR

Sample Type(s): Feces

Input: Up to 100mg of sample

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.

Further information can be found in Technical Manual #TM330, available at: www.promega.com/protocols

or by e-mailing technical services at techserv@promega.com

Materials Required:

- CTAB Buffer, homemade or MC1411
- 100% Isopropanol
- Heat block capable of holding temperatures of up to 95°C
- Disposable pestle (i.e.: 12-141-363 from Fisher Scientific) or other homogenization method such as bead beating (optional)

Protocol:

**Important, pre-warm elution buffer to 70°C.

1. Place up to 250mg of sample (50-100mg is a good starting amount) into a 2ml microcentrifuge tube (click fit or screw top is preferred).
2. Add 1ml of CTAB Buffer and vortex for 30 seconds
3. Heat sample at 95°C for 5 minutes
4. Vortex thoroughly for 30 seconds-1 minute, until large solids are mostly dispersed.
5. If necessary, manually homogenize with disposable pestle or other preferred technique (For example, bead beating 30 sec). Very dry or frozen samples may need this step. Some small intact pieces are OK especially with high fecal input.
6. Add 40µl of Proteinase K and vortex to mix.
7. Incubate sample at 70°C 10 minutes.
8. Spin 5 minutes at max speed.
9. Carefully remove 300µl of supernatant and transfer to a clean 1.5ml microcentrifuge tube.
10. Add 10µl of RNase A and incubate 5 minutes at room temperature. (optional)
11. Add 300µl of CLD Buffer to supernatant. Gently mix by inversion.
12. Add 600µl of 100% isopropanol and gently invert to mix.
13. Load 600µl of sample to a ReliaPrep™ Binding Column placed in a collection tube, spin for 1 minute at max speed. Discard flow through.
14. Load the rest of sample to the ReliaPrep™ Binding Column, spin for 1 minute at max speed. Place Binding Column into a new collection tube.
15. Add 500µl of Column Wash Buffer (CWD).

16. Spin 2 minutes at max speed and then discard flow through.
17. Repeat steps 15 and 16 twice, for a total of three washes.
18. Place Binding Column in a labeled elution tube. Add 100-200µl of pre-warmed Elution Buffer to Binding Column. Spin 1 minute at max speed.

Example Results:

Nucleic acid was isolated from 50 mg human fecal samples spiked with neat, 1:10 or 1:100 diluted Zika virus or CMV using modified method for the ReliaPrep™ Blood gDNA MiniPrep System outlined above. Following nucleic acid isolation, viral recovery was measured by amplification using virus specific primer and probe sets using GoTaq® amplification reagents. Recovery of DNA and RNA virus was efficiently performed with ReliaPrep™ (Figure 1).

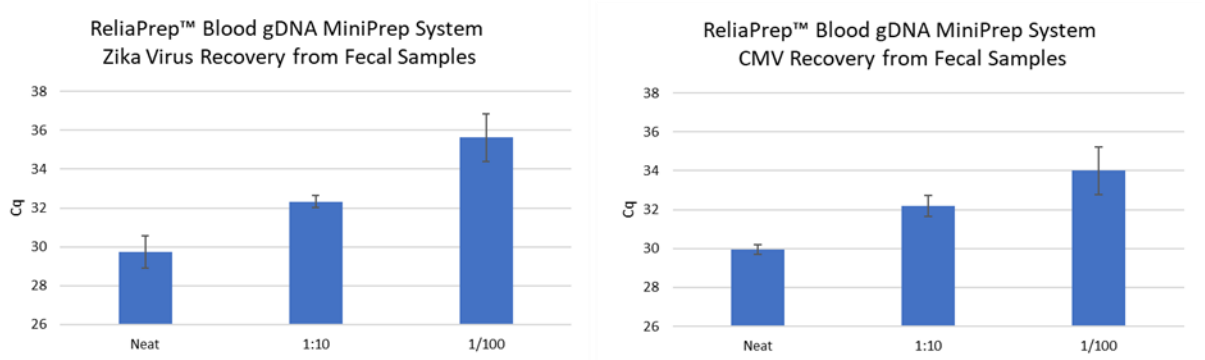

Figure 1. Viral nucleic acid recovery from feces with the Maxwell® RSC. Left: Ct values from RT-qPCR to detect Zika virus RNA recovered from feces. Right: Ct values from qPCR to detect CMV DNA recovered from feces. N=3 for each condition.