Promega

Product Application

Viroid RNA extraction from lemon leaves using Maxwell® RSC

RNA of viroid was extracted from lemon leaves using Maxwell® RSC miRNA Tissue Kit

Kit: Maxwell® RSC miRNA Tissue Kit, Cat. #AS1460

Analyses: RT-qPCR

Sample Type(s): Lemon leaves infected with Citrus Exorcortis viroid[1] (CEVd)

Input: 450 µl of homogenate

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 #TM441, available at: **www.promega.com/protocols**

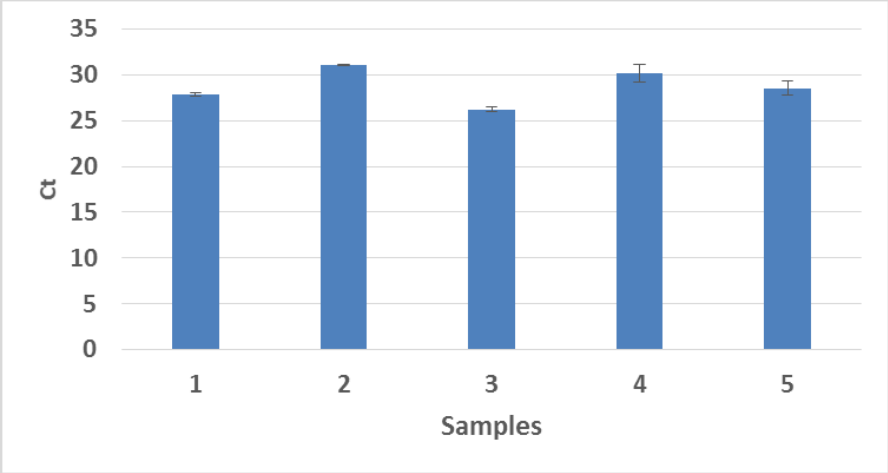
Materials Required:

- Maxwell® RSC instrument, Cat. #AS4500
- Maxwell® RSC miRNA Tissue Kit, Cat. AS1460
- CEVd One-step Real-Time RT-PCR (Qualiplante[1], Ref: SYBR.CEVd-100Liq)
- Mortar and pestle
- Centrifuge

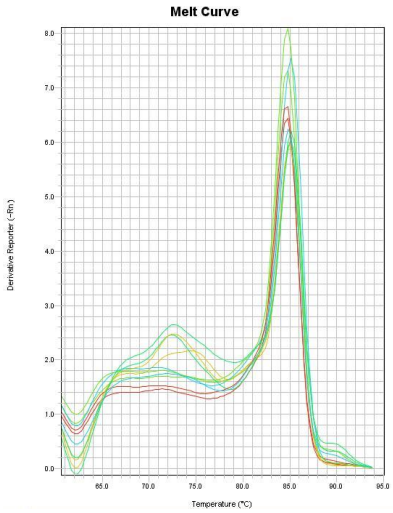
Protocol:

1. Grind 1 g of small pieces of leaf with a precooled mortar and pestle
2. Add 4.5 ml of precooled 1-Thioglycerol/Homogenization buffer and mix by vortexing.
3. Centrifuge for 5 min at maximum speed to recover the homogenate.
4. Add 450 µl of homogenate in a 1.5 ml tube.
5. Add 450 µl of Lysis buffer + 30 µl of proteinase K. Mix by vortexing for 20 seconds
6. Incubate at room temperature for 10 minutes. During this time, prepare the Maxwell® RSC Cartridges.
7. Transfer 400µl of lysate to well #1 of the Maxwell® RSC Cartridge. Add 10µl of blue DNase I Solution to well #4.
8. Place a Maxwell® RSC Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
9. Place 0.5ml elution Tubes in the front of the deck tray. Add 60µl of Nuclease-Free Water to the bottom of each elution Tube.
10. Run RSC miRNA Tissue method on Maxwell® RSC Instrument.

Results:



RT-qPCR amplification. Ct values for amplification of RNA extracted using the Maxwell® RSC miRNA Tissue Kit from 5 different lemon leaves infected by CEVd (1 to 5). RT-qPCR were performed using CEVd One-step Real-Time RT-PCR (Qualiplant, Ref: SYBR.CEVd-100Liq) which amplify specifically RNA from CEVd. Positive and negative controls are validated according with the instructions of the manufacturer.



RT-qPCR amplification. Melting curve for amplification of RNA extracted using the Maxwell® RSC miRNA Tissue Kit from 5 different lemon leaves infected by CEVd. The analysis of melting curve for RNA samples extracted using the Maxwell® RSC miRNA Tissue shows the specific peak of amplification at ~85°C as indicated in the manufacturer's protocol.

[1] Acknowledgements: IpadLab srl (Lodi, Italy) for the samples and Qualiplante SAS (Clapiers, France) for the kit.