Promega

Product Application

Purification of *Xylella fastidiosa* DNA from Infected Plant Tissue Using the KingFisher™ Flex

Purify Xylella fastidiosa DNA from infected plant tissue using the Maxwell® HT 96 gDNA Blood Isolation System on the KingFisher™ Flex Purification System.

Kit: Maxwell® HT 96 gDNA Blood Isolation System (Cat.# A2670)

Analyses: qPCR

Sample Type: Plant tissue

Input: 1g of Leaf midribs, petioles and twigs

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 TM368, available at: **www.promega.com/protocols**

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

Materials Required:

- KingFisher™ Flex Purification System (Thermo Fisher Scientific, Cat.# 5400630)
- KingFisher™ deep-well 96 Plate (Thermo Fisher Scientific, Cat.# 95040450)
- KingFisher™ 96 Tip Comb for DW Magnets (Thermo Fisher Scientific, Cat.# 97002534)
- Maxwell® HT 96 gDNA Blood Isolation System (Cat.# A2670)
- CTAB Buffer (Cat.# MC1411)
- 50% Ethanol
- 100% Isopropanol
- Bioreba extraction bag (Cat.# 430100, Bioreba)
- Hammer
- Heat block set to 65°C
- Microcentrifuge

Protocol:

1. Add 1g of Leaf midribs, petioles and twigs in a Bioreba extraction bag.
2. Add 5ml of CTAB and homogenize with a hammer to grind the tissues.
3. Transfer 1ml of lysate into 1.5ml tube. **Note:** Avoid transferring solid material
4. Add 40µl Proteinase K and 20µl RNase A Solution per tube.
5. Vortex for 10 seconds.
6. Incubate in a heat block at 65°C for 30 minutes.
7. Prepare 96-well Deep Well plates during the incubation, as indicated below:
    - Plate 1 = Tip Plate
        - Add KingFisher™ 96 Tip Comb for DW Magnets.
    - Plate 2 = Elution Plate
        - Add 110µl of Elution Buffer per well.
    - Plate 3 = 50% EtOH
        - Add 450µl 50% EtOH per well (make fresh daily).
    - Plate 4 = Wash 3
        - Add 400µl Wash Buffer (WBA) and 50µl 50% EtOH per well.

- Plate 5 = Wash 2
  - Add 900µl Wash Buffer (WBA) and 100µl 50% EtOH per well.
- Plate 6 = Wash 1
  - Add 900µl Wash Buffer (WBA) and 100µl 50% EtOH per well.
- Plate 7 = Bind Plate
  - Add 365µl of 100% Isopropanol, 300µl Cell Lysis Buffer (CLD), and 35µl of Resin per well. **Note:** Vortex/shake resin thoroughly to resuspend before addition.

8. Following incubation, vortex each plant tissue sample for 10 seconds.
9. Centrifuge plant tissue samples for 10 minutes at ≥ 16,000 x g.
10. For each plant tissue sample, transfer 300µl of clear lysate per well into the necessary wells of the Bind Plate (Plate 7). Avoid transferring solid material and oil as these materials can inhibit downstream assays.
11. Load the plates onto the KingFisher™ Flex Purification System and run the method "PureFood_96_V1.0".

Results: DNA was successfully purified from *Xylella fastidiosa* spiked plant tissue samples using the Maxwell® HT 96 gDNA Blood Isolation System Kit on the KingFisher™ Flex Purification System. *Xylella fastidiosa* DNA was specifically detected via probe qPCR.

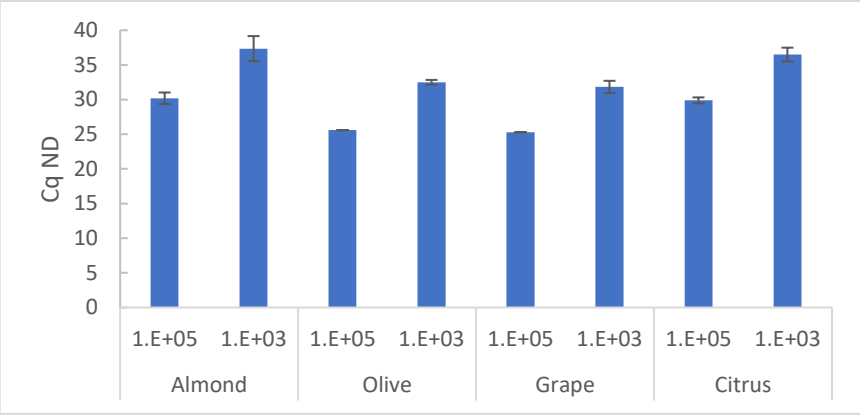

Figure 1. Cq values from probe qPCR amplification of DNA purified from *Xylella fastidiosa* spiked plant tissue using the Maxwell® HT 96 gDNA Blood Isolation System Kit on the KingFisher™ Flex Purification System. DNA was purified from 1g of almond, olive, grape and citrus leaf midribs, petioles and twigs. Lysates were spiked with 10^5 or 10^3 CFU/100µl of *Xylella fastidiosa* prior to extraction. 2µl of undiluted DNA (ND) was amplified in a 20µl reaction using GoTaq® Probe qPCR Master Mix (Cat.# A6101) and *X. fastidiosa* primers and probe (Harper et al. 2010) according to the EPPO 2019 guidelines[1]. NIC (negative isolation controls) were run in parallel for each plant tissue and all of them did not amplify. Results are mean ± STD (N=2 extractions per spiked sample, N=1 amplification per eluate).

References:

1. European and Mediterranean Plant Protection Organization. (2019). Bulletin OEPP/EPPO Bulletin: PM 7/24 (4) *Xylella fastidiosa*. 49 (2), 175–227.