TECHNICAL SHEET No. 16

Virus Detection: *Potato leaf roll virus* (PLRV)

Method: Immunocapture RT-PCR

**General**
Virus detected: PLRV from potato leaf and potato tubers.
General method was immunocapture RT-PCR

**Developed by**
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**Goals**
To develop a sensitive method for PLRV detection based on immunocapture RT-PCR.

**Methods**

**Immunocapture IC**
1. Add 200 μl of anti-PLRV antibody (diluted 1:1000 in coating buffer) to each ELISA plate well or to PCR tube and incubate at 37°C for 3 h. We used a commercial antibody (Bioreba).
2. Empty the ELISA well/ PCR tube and wash 3 times with TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween-20).
3. Homogenize 25 mg of plant leaf or tuber with 5 ml of suitable extraction buffer.
4. Add 200 μl of the homogenate to the coated ELISA well/ PCR tube and incubate for 18 h at 4 °C.
5. Empty the ELISA well/ PCR tube and wash 3 times with TBST.
6. Dry the ELISA well/ PCR tube, add 5 to10 μl ddH₂O and heat at 70 °C for 15 min.

**RT-PCR**

*RNA synthesis*
1. To 5 μl IC add 8 μl ddH₂O and 1 μl primer C582 (100 pmoles); incubate at 70 °C for 10 min, followed by 30 min in and ice bath.
2. Add 1 μl of each dNTPs (25mM each), 4 μl reverse transcriptase buffer 5X, 1 μl AMV reverse transcriptase (Promega); incubate at 42 °C for 1h.
3. Heat for 10 min at 90 °C; adjust the volume to 50 μl with ddH₂O

*PCR*
The primers used were as follows (derived from sequence GenBank accession numbers D13954 and D13953):

C582: 5’TATCGTCCATGGGTACGGTCGTGGT3’
R732: 5’TCTAGATCTTTGGGTTTTGCAAAGC3’

Following PCR, a 640 bp band is obtained.
1. The PCR reaction contains 5 μl from the reverse transcriptase reaction, 0.25 μl 25mM dNTPs, 1 μl each primers 1 (100 pmoles each), 2.5 μl Taq 10 x buffer and 1 unit *Taq* polymerase; add ddH₂O to a final volume of 25 μl.
2. Cycling: One cycle: 95 °C for 3 min, 50 °C for 2 min, 72 °C for 2 min. Thirty cycles: 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min. One additional cycle: 72 °C for 10 min.

3. Analysis of reaction products: Subject the reaction products to 1% agarose gel electrophoresis.

**Results**

The following figure shows the detection of PLRV by IC-RT-PCR and by RT-PCR. For RT-PCR (lanes 1 – 3), RNA was from a *Datura stramonium* infected plant. For IC-RT-PCR (lanes 1’ - 3’), samples were from a *Datura stramonium* infected plant. M: molecular weight marker; P: cloned PLRV DNA; 0: no template.