

USDA-APHIS-PPQ-S&T-CPHST - Beltsville Laboratory
Pospiviroid Generic Conventional Master Mix Form

Reference WI-B-T-1-64
Extraction WI Reference: WI-B-T-2-16

Test	Sample
	QC
1	19102201-01
2	19102202-01
3	19102203-01
4	19102204-01
5	19102205-01
6	19102206-01
7	NTC
8	Viroid
9	Healthy Seed Control
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Equipment	
PCR Room	Select
Diagnostics Room	Select
Conventional Thermal Cycler	Select
Tapestation	Select

Reference Form-B-20 for current equipment calibration dates

RT-PCR Master Mix for Pospiviroid (generic primer) and CLVd (specific primer)				
Master Mix	1 sam. (µl)	Sam. #	CHECK	Lot/Batch #
Molc. Grade water	20.00	198.00		
2x buffer	25.00	247.50		
Forward primer/Reverse primer mix (10 µM mix)	1.00	9.90		
SuperScript III RT/Platinum Taq Mix (2U/µL)	2.00	19.80		
Master Mix	48.00			
			Pos Control	

PCR Master Mix for Internal Control Nad5 (specific for plant genome)				
Master Mix	1 sam. (µl)	Sam. #	CHECK	Lot/Batch #
Molc. Grade water	20.00	198.00		
2x buffer	25.00	247.50		
PNAD 5f & PNad5.656.F Complement Primer r	1.00	9.90		
SuperScript III RT/Platinum Taq Mix (2U/µL)	2.00	19.80		
Master Mix	48.00			
			Pos Control	

Notes: Add 2.00 µl of sample/control

ADDITIONAL INFORMATION:

PCR Parameters:

Step 1: 50°C, 30 Minutes	Step 4: 30 CYCLES OF THE FOLLOWING
Step 2: 94°C, 1:30 Minutes	A: 94°C, 30 Sec
Step 3: 15 CYCLES OF THE FOLLOWING	B: 59°C, 90 Sec
A: 94°C, 30 Sec	C: 72°C, 45 Sec
B: 62°C, 90 Sec	Step 5: Extension 72°C, 7 min
C: 72°C, 45 Sec	Step 6: 4°C, Hold

**Use Maximum ramping rate*

	Lot Number
Tapestation Ladder	
Tapestation Buffer	
ScreenTape	

Tapestation Note: Add 1 µL of sample or ladder to 3 µL of buffer

Notes: Record any other equipment used here, etc.