

USDA-APHIS-PPQ-S&T-CPHST - Beltsville Laboratory

Reference: WI-B-T-2-16

PospiA qPCR Standard Master Mix on Quant 7

Well	Sample Number
	QC
1	19102201-01
13	19102201-02
25	19102201-03
61	19102202-01
73	19102202-02
85	19102202-03
2	19102203-01
14	19102203-02
26	19102203-03
62	19102204-01
74	19102204-02
86	19102204-03
3	19102205-01
15	19102205-02
27	19102205-03
63	19102206-01
75	19102206-02
87	19102206-03
4	NTC
16	Healthy Seed Control
76	PSTVd Pos Control
88	PCFVd Pos Control

PospiA Master Mix				
rMix	Per sample (µl)	Sam. #	CHECK	Lot Number
		22		
Rnase-free water	11.50	278.30	✓	
Ultraplex 1-Step Tough Mix	6.25	151.25	✓	
10 uM Pospi A primer mix	0.75	18.15	✓	
10 uM Pospi A probe mix	0.50	12.10		
Total Volume	19.00			
		PSTVd control		
		PCFVd control		

Pospi Real-Time PCR Parameters:
Hold Stage: 1.) Hold at 50°C with Data Collection OFF
 2.) Hold at 95°C for 3 min with Data Collection OFF
**Use Instrument Automatic Ramp rate of 2.445 degrees per second.*
PCR Stage 40 CYCLES (AutoDelta disabled)
 Step 1.) 95°C for 10 seconds Data Collection OFF
 Step 2.) 60°C for 1 minute Data Collection ON
**Instrument Automatic ramp 1.936 degrees per second.*

Equipment	
PCR Room	Select
Diagnostics Room	Select
Real-Time Thermal Cycle	Select

Reference Form-B-20 for current equipment calibration

PCR Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	19102201-01	19102203-01	19102205-01	NTC								
B	19102201-02	19102203-02	19102205-02	Healthy Seed Control								
C	19102201-03	19102203-03	19102205-03									
D												
E												
F	19102202-01	19102204-01	19102206-01	PSTVd Pos Control								
G	19102202-02	19102204-02	19102206-02	PCFVd Pos Control								
H	19102202-03	19102204-03	19102206-03									

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Approved by: Nicole O'Donahue

Effective Date: 9/30/2019

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PospiB qPCR Standard Master Mix on Quant 7

Reference: WI-B-T-2-16

Well	Sample Number
	QC
5	19102201-01
17	19102201-02
29	19102201-03
65	19102202-01
77	19102202-02
89	19102202-03
6	19102203-01
18	19102203-02
30	19102203-03
66	19102204-01
78	19102204-02
90	19102204-03
7	19102205-01
19	19102205-02
31	19102205-03
67	19102206-01
79	19102206-02
91	19102206-03
8	NTC
20	Healthy Seed Control
92	CEVd Pos Control

PospiB Master Mix			
rMix	Per sample (µl)	Sam. #	Lot Number
		21	
Rnase-free water	11.50	265.65	
Ultrplex 1-Step Tough Mix	6.25	144.38	
10 uM Pospi B primer mix	0.75	17.33	
10 uM Pospi B probe mix	0.50	11.55	
Total Volume	19.00		
			Pos Control

Pospi Real-Time PCR Parameters:
Hold Stage: 1.) Hold at 50°C with Data Collection OFF
 2.) Hold at 95°C for 3 min with Data Collection OFF
**Use Instrument Automatic Ramp rate of 2.445 degrees per second.*
PCR Stage 40 CYCLES (AutoDelta disabled)
 Step 1.) 95°C for 10 seconds Data Collection OFF
 Step 2.) 60°C for 1 minute Data Collection ON
**Instrument Automatic ramp 1.936 degrees per second.*

Equipment	
PCR Room	Select
Diagnostics Room	Select
Real-Time Thermal Cycle	Select

Reference Form-B-20 for current equipment calibration dates

Pospiviroid B qPCR Master Mix Form

PCR Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A				19102201-01	19102203-01	19102205-01	NTC					
B				19102201-02	19102203-02	19102205-02	Healthy seed Control					
C				19102201-03	19102203-03	19102205-03						
D												
E												
F				19102202-01	19102204-01	19102206-01						
G				19102202-02	19102204-02	19102206-02						
H				19102202-03	19102204-03	19102206-03	CEVd Pos Control					

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Pospiviroid C qPCR Master Mix Form

USDA-APHIS-PPQ-S&T-CPHST - Beltsville Laboratory

Reference: WI-B-T-1-63

Pospic qPCR Standard Master Mix on Quant 7

Well	Sample Number
	QC
9	19102201-01
21	19102201-02
33	19102201-03
69	19102202-01
81	19102202-02
93	19102202-03
10	19102203-01
22	19102203-02
34	19102203-03
70	19102204-01
82	19102204-02
94	19102204-03
11	19102205-01
23	19102205-02
35	19102205-03
71	19102206-01
83	19102206-02
95	19102206-03
12	NTC
24	Healthy Seed Control
96	TPMvd Pos Control

Pospic Master Mix				
rMix	Per sample (µl)	Sam. #	CHECK	Lot Number
Rnase-free water	11.50	265.65	✓	
Ultrplex 1-Step Tough Mix	6.25	144.38	✓	
10 uM Pospic C primer mix	0.75	17.33	✓	
10 uM Pospic C probe mix	0.50	11.55	✓	
Total Volume	19.00			
		Pos Control		

Pospic Real-Time PCR Parameters:
Hold Stage: 1.) Hold at 50°C with Data Collection OFF
 2.) Hold at 95°C for 3 min with Data Collection OFF
**Use Instrument Automatic Ramp rate of 2.445 degrees per second.*
PCR Stage 40 CYCLES (AutoDelta disabled)
 Step 1.) 95°C for 10 seconds Data Collection OFF
 Step 2.) 60°C for 1 minute Data Collection ON
**Instrument Automatic ramp 1.936 degrees per second.*

Equipment	
PCR Room	Select
Diagnostics Room	Select
Real-Time Thermal Cycle	Select

Reference Form-B-20 for current equipment calibration dates

Pospiviroid C qPCR Master Mix Form

PCR Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A								19102201-01	19102203-01	19102205-01	NTC	
B								19102201-02	19102203-02	19102205-02	Healthy seed Control	
C								19102201-03	19102203-03	19102205-03		
D												
E												
F								19102202-01	19102204-01	19102206-01		
G								19102202-02	19102204-02	19102206-02	TPMVD Pos Control	
H								19102202-03	19102204-03	19102206-03		

The correct revision of this document is located at I:\CPHST1-BV\MM

Approved by: Nicole O'Donahue

Effective Date: 9/30/19

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Reference: WI-B-T-1-63

Pospit qPCR Standard Master Mix on Quant 7

Well	Sample Number
	QC
1	19102201-01
13	19102201-02
25	19102201-03
61	19102202-01
73	19102202-02
85	19102202-03
2	19102203-01
14	19102203-02
26	19102203-03
62	19102204-01
74	19102204-02
86	19102204-03
3	19102205-01
15	19102205-02
27	19102205-03
63	19102206-01
75	19102206-02
87	19102206-03
4	NTC
16	Healthy Seed Control
28	TASVd Pos Control

Pospit Master Mix			
rMix	Per sample (µl)	Sam. #	Lot Number
Rnase-free water	10.75	248.33	
Ultrplex 1-Step Tough Mix	6.25	144.38	✓
10 uM Pospit TASVd-F2-200	0.75	17.33	✓
10 uM Pospit TASVd-R2-269	0.75	17.33	✓
10 uM Pospit TASVd-P2-228	0.50	11.55	✓
Total Volume	19.00		
Pos Control			

Pospit Real-Time PCR Parameters:
Hold Stage: 1.) Hold at 50°C with Data Collection OFF
 2.) Hold at 95°C for 3 min with Data Collection OFF
**Use Instrument Automatic Ramp rate of 2.445 degrees per second.*
PCR Stage 40 CYCLES (AutoDelta disabled)
 Step 1.) 95°C for 10 seconds Data Collection OFF
 Step 2.) 60°C for 1 minute Data Collection ON
**Instrument Automatic ramp 1.936 degrees per second.*

Equipment	
PCR Room	Select
Diagnostics Room	Select
Real-Time Thermal Cycle	Select

Reference Form-B-20 for current equipment calibration dates

Pospiviroid T qPCR Master Mix Form

PCR Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	19102201-01	19102203-01	19102205-01	NTC								
B	19102201-02	19102203-02	19102205-02	Internal Seed Control								
C	19102201-03	19102203-03	19102205-03	TRAVERTUS Control								
D												
E												
F	19102202-01	19102204-01	19102206-01									
G	19102202-02	19102204-02	19102206-02									
H	19102202-03	19102204-03	19102206-03									

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