Validation Report: PCR Primers

PCR 93000 • *Carlavirus* Group (Carla)

Test Characteristics

Test Name	Carlavirus Group	**Format**	RT-PCR Primers
Catalog Number	93000	**Extraction Method**	Nucleic acid extraction
Acronym	Carla		
Genus	Carlavirus		

Summary

The Carlavirus Group PCR primers offer a sensitive diagnostic method to detect members of the Carlavirus genus of the Betaflexiviridae family. The primer sequences are based on conserved genome regions and can detect characterized and unassigned members of the Carlavirus genus.

Diagnostic Sensitivity

True Positives	1006
Correct Diagnoses	1006
Percent	100%

Analytical Specificity

Inclusivity:

Carlaviruses[1] Detected:

Virus Name	Species Name
Aconitum latent virus (AcLV)[2]	Carlavirus latensaconiti
American hop latent virus (AHLV)[2]	Carlavirus americanense
Atractylodes mottle virus (AtrMoV)[2]	Carlavirus atractylodis
Blueberry scorch virus (BlScV)	Carlavirus vaccinii
Butterbur mosaic virus (ButMV)[2]	Carlavirus petasitis
Cactus virus 2 (CV-2)[2]	Carlavirus cacti
Caper latent virus (CapLV)[2]	Carlavirus latenscapparis
Carnation latent virus (CLV)	Carlavirus latensdianthi
Chrysanthemum virus B (CVB)	Carlavirus betachrysanthemi
Cole latent virus (CoLV)[2]	Carlavirus latensbrassicae
Coleus vein necrosis virus (CVNV)	Carlavirus colei
Cowpea mild mottle virus (CPMMV)	Carlavirus vignae
Cucumber vein-clearing virus (CuVCV)[2]	Carlavirus cucumis
Daphne virus S (DVS)[2]	Carlavirus sigmadaphnis
Gaillardia latent virus (GaLV)[2]	Carlavirus latensgaillardiae
Garlic common latent virus (GarCLV)	Carlavirus latensallii
Helleborus mosaic virus (HeMV)	Carlavirus hellebori

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Carlaviruses[1] Detected:

Virus Name	Species Name
Helleborus net necrosis virus (HeNNV)	Carlavirus necroretis
Hippeastrum latent virus (HLV)[2]	Carlavirus latenshippeastri
Hop latent virus (HpLV)	Carlavirus latenshumuli
Hop mosaic virus (HpMV)[2]	Carlavirus humuli
Hydrangea chlorotic mottle virus (HdCMV)	Carlavirus hydrangeae
Kalanchoe latent virus (KLV)	Carlavirus latenskalanchoe
Ligustrum necrotic ringspot virus (LNRSV)	Carlavirus necroligustri
Ligustrum virus A (LVA)[2]	Carlavirus alphaligustri
Lily symptomless virus (LSV)	Carlavirus lilii
Melon yellowing-associated virus (MYaV)[2]	Carlavirus melonis
Mirabilis jalapa mottle virus (MjMV)[2]	Carlavirus mirabilis
Narcissus common latent virus (NCLV)[2]	Carlavirus latensnarcissi
Nerine latent virus (NeLV)	Carlavirus latensnerinis
Passiflora latent virus (PLV)[2]	Carlavirus latenspassiflorae
Pea streak virus (PeSV)	Carlavirus pisi
Phlox virus B (PhlVB)[2]	Carlavirus betaphlocis
Phlox virus M (PhlVM)	Carlavirus miphlocis
Phlox virus S (PhlVS)[2]	Carlavirus sigmaphlocis
Poplar mosaic virus (PopMV)[2]	Carlavirus populi
Potato latent virus (PotLV)	Carlavirus latensolani
Potato virus H (PVH)[2]	Carlavirus chisolani
Potato virus M (PVM)	Carlavirus misolani
Potato virus P (PVP)[2]	Carlavirus pisolani
Potato virus S (PVS)	Carlavirus sigmasolani
Red clover vein mosaic virus (RCVMV)	Carlavirus trifolii
Sambucus virus C (SVC)[2]	Carlavirus gammasambuci
Sambucus virus D (SVD)[2]	Carlavirus deltasambuci
Sambucus virus E (SVE)[2]	Carlavirus epsilonsambuci
Shallot latent virus (SLV)	Carlavirus latensascalonici
Sint-Jan onion latent virus (SJOLV)[2]	Carlavirus cornutum
Strawberry pseudo mild yellow edge virus (SPMYEV)[2]	Carlavirus fragariae
Sweet potato C6 virus (SC6V)[2]	Carlavirus ipomoeae
Sweet potato chlorotic fleck virus (SPCFV)[2]	Carlavirus chloroipomoeae
Verbena latent virus (VeLV)[2]	Carlavirus latensverbenae
Yam latent virus (YLV)[2]	Carlavirus latensdioscoreae

[1]The list above represents viruses that have been shown to be detected by this group PCR test. It also represents viruses that may be detected based on *in silico* analysis. If you have confirmed detection of a predicted virus detection or a virus not on this list, please contact us. We would like to work with you to further validate detection capabilities.

[2]Predicted detection by *in silico* analysis only

Carlaviruses Not Detected:

None Known	

Exclusivity:

Cross-reacts With:

Virus Name	Species Name
None Known	

Does Not Cross-react With:

Virus Name	Species Name
None Known	

Diagnostic Specificity

True Negatives	3935
Correct Diagnoses	3935
Percent	100%

Selectivity:

No Matrix Effect Observed With:			
Achillea leaves	Agapanthus leaves	Agastache leaves	Allium leaves
Althernantera leaves	Amsonia leaves	Anemone leaves	Angelonia leaves
Artemisia leaves	Aster leaves	Astilbe leaves	Astilbe roots
Bean leaves	Blackberry leaves	Blueberry leaves	Brunnera leaves
Buddleia leaves	Calamintha leaves	Campanula leaves	Canna leaves
Catharanthus leaves	Centauria leaves	Chile pepper leaves	Cimicifuga leaves
Clematis leaves	Coleus leaves	Colocasia leaves	Coreopsis leaves
Cowpea leaves	Crinum leaves	Crisantemo leaves	Crossandra leaves
Daphne leaves	Delosperma leaves	Delphinium leaves	Dianthus leaves
Dicentra leaves	Dicentra leaves and midrib	Echinacea leaves	Epimedium leaves
Escallonia leaves	Garlic cloves	Gentiana leaves	Gypsophila leaves
Gypsophila paniculata leaves	Helebore leaves	Heliopsis leaves	Hellebore leaves
Helleborus leaves	Hemp leaves	Hesperozygis leaves	Heuchera leaves
Hibiscus leaves	Hops leaves	Hosta leaves	Hosta roots
Hydrangea leaves	Impatiens leaves	Ipomoea leaves	Iris leaves
Knautia leaves	Lagerstroemia leaves	Lavandula leaves	Leucanthemum leaves
Ligularia leaves	Lily leaves	Limonium leaves	Lobelia leaves
Mandevilla leaves	Molinia leaves	Monarda leaves	Nepeta leaves
Origanum leaves	Passiflora leaves	Passionflower leaves	Penstemon leaves
Pepper leaves	Perovskia leaves	Philadelphus leaves	Phlox leaves
Polemonium leaves	Potato leaves	Raspberry leaves	Rubus leaves
Rudbeckia leaves	Salvia leaves	Schlumbergera leaves	Sedum leaves
Sedum roots	Sempervivum leaves	Silene leaves	Soybean leaf
Soybean leaves	Soybean pods	Soybean seeds	Soybean stems
Spigelia leaves	Statice leaves	Strawberry leaves	Sweet potato leaves
Syringa leaves	Thymus leaves	Tobacco leaves	Tomato leaves

Tradescantia leaves	Verbena leaves	Vernonia leaves	Veronica leaves
Vitex leaves			

The hosts on the above list have been chosen to represent those which historically cause a range of matrix effects, in addition to those expected to be screened for this pathogen. Not all plant species susceptible to this pathogen have been screened, but may still be used with this assay unless otherwise noted below. As with all diagnostic tools, Agdia recommends confirming all results with a secondary detection method before making any economic decisions (ex: discarding plants due to positive test results, etc.).

Matrix Effect Observed With:			
None Known			

Robustness

Planned deviation analysis:
No deviations from the user guide protocol were validated.

Stability:

	1-year stability (accelerated)	Real-time Stability Verification
Positive Sample (High)	Pass	Monitoring
Positive Sample (High)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Positive Sample (Low)	Pass	Monitoring
Negative Sample	Pass	Monitoring
Negative Sample	Pass	Monitoring
Negative Sample	Pass	Monitoring

Glossary

Diagnostic sensitivity[1]: The percentage of positive samples correctly identified in an experiment with known positive controls.

Diagnostic specificity[1]: The percentage of negative samples correctly identified in an experiment with known negative controls.

Analytical sensitivity[3]: The smallest amount of target that can be detected reliably (this is sometimes referred to as the 'limit of detection')

Analytical specificity[3]: (comprises inclusivity and exclusivity)

Inclusivity[3]: The performance of a test with a range of target isolates covering genetic diversity, different geographical origin and/or hosts associated with the target organism.

Exclusivity[3]: The performance of a test with a range of non-targets (e.g. cross-reaction with closely related organisms, contaminants)

Selectivity[2]: The level of effect that matrices and relevant plant parts have on the performance of the assay.

Repeatability[2]: The agreement between test replicates of the same sample tested by the same operator.

Reproducibility[3]: The ability of a test to provide consistent results when applied to aliquots of the same sample tested under different conditions (e.g. time, users, equipment, location)

Robustness[1,3]: The extent to which varying test conditions (e.g. temperature, volume, change of buffers) affect the established test performance values. May also be referred to as planned deviation analysis.

Stability[1]: The performance of test reagents or controls over time.

References:
[1]Groth-Helms, D., Rivera, Y., Martin, F. N., Arif, M., Sharma, P., Castlebury, L. A. (in press). Terminology and Guidelines for Diagnostic Assay Development and Validation: Best Practices for Molecular Tests. PhytoFrontiers.
[2]Eads, A., Groth-Helms, D., Davenport, B., Cha, X., Li, R., Walsh, C., Schuetz, K., (in press). The Commercial Validation of Three Tomato Brown Rugose Fruit Virus Assays. PhytoFrontiers.
[3]EPPO (2018) PM 7/76 (5) Use of EPPO Diagnostic Standards, EPPO Bulletin 48, 373– 377.