AmplifyRP® XRT for *Fo*
Validation Report
Fusarium oxysporum
Product No. XCS 70500

Test Characteristics

Test Name	Fusarium oxysporum	**Test Label**	FAM-labeled target probe
Catalog Number	70500	**Internal Control**	ROX-labeled control probe (endogenous)
Acronym	Fo	**Format**	XRT
Genus	Fusarium	**Diluents**	GEB/PD1
		Sample Dilution	1:20

Summary

AmplifyRP® XRT for Fo is a rapid DNA amplification and detection platform designed for testing cannabis plants for Fusarium oxysporum. This kit includes lyophilized reaction pellets containing the necessary reagents to amplify Fo DNA and an endogenous DNA control at a single operating temperature (42 °C).

Diagnostic Sensitivity

True Positives	84
Correct Diagnoses	82
Percent	97.6%

Analytical Sensitivity

Limit of Detection: Approximately 32 - 72 copies of DNA fragments

Analytical Specificity

Inclusivity:

Formae Speciales Detected:

Fusarium oxysporum f.sp. albedinis[1]	Fusarium oxysporum f.sp. asparagi[1]
Fusarium oxysporum f.sp. batatas	Fusarium oxysporum f.sp. canariensis[1]
Fusarium oxysporum f.sp. cannabis	Fusarium oxysporum f.sp. cattleyae[1]
Fusarium oxysporum f.sp. cepae[1]	Fusarium oxysporum f.sp. chrysanthemi
Fusarium oxysporum f.sp. ciceris[1]	Fusarium oxysporum f.sp. cubense
Fusarium oxysporum f.sp. cucumerinum[1]	Fusarium oxysporum f.sp. cyclaminis
Fusarium oxysporum f.sp. dianthi[1]	Fusarium oxysporum f.sp. fragariae
Fusarium oxysporum f.sp. gladioli[1]	Fusarium oxysporum f.sp. koae[1]
Fusarium oxysporum f.sp. lactucae[1]	Fusarium oxysporum f.sp. lentis[1]
Fusarium oxysporum f.sp. lilli	Fusarium oxysporum f.sp. lini
Fusarium oxysporum f.sp. lycopersici	Fusarium oxysporum f.sp. medicaginis[1]
Fusarium oxysporum f.sp. melonis	Fusarium oxysporum f.sp. narcissi[1]
Fusarium oxysporum f.sp. nicotianae[1]	Fusarium oxysporum f.sp. niveum[1]
Fusarium oxysporum f.sp. palmarum[1]	Fusarium oxysporum f.sp. passiflorae
Fusarium oxysporum f.sp. phaseoli[1]	Fusarium oxysporum f.sp. pisi[1]
Fusarium oxysporum f.sp. radicis-lycospersici	Fusarium oxysporum f.sp. tuberosi[1]
Fusarium oxysporum f.sp. tuliape[1]	Fusarium oxysporum f.sp. vasinfectum
Fusarium oxysporum f.sp. vasinfectum[1]	Fusarium oxysporum f.sp. zingiberi[1]
[1]Predicted detection by *in silico* analysis only	

Formae Speciales Not Detected:

Fusarium oxysporum f.sp. betae	

Exclusivity:

Cross-reacts With:

Fusarium sporotrichioides[1]	
[1]A weak cross reaction was observed inconsistently.	

Does Not Cross-react With:

Alfalfa mosaic virus (AMV)[1]	Alternaria alternata
Anthracnose spp.[1]	Armillaria mellea[1]
Aspergillus[1]	Beet curly top virus (BCTV)
Botryosphaeria dothidea[1]	Botryosphaeria stevensii[1]
Botrytis cinerea[1]	Bremia lactucae[1]
Colletotrichum actuatum[1]	Colletotrichum gloeosporioides
Diplodia seriata[1]	Fusarium anthophilum[1]
Fusarium avenaceum[1]	Fusarium brachygibbosum
Fusarium chlamydosporum[1]	Fusarium culmorum[1]
Fusarium equiseti[1]	Fusarium fujikuroi[1]
Fusarium graminearum	Fusarium langsethiae[1]
Fusarium maniliforme	Fusarium meridionale[1]
Fusarium pallidoroseum	Fusarium poae[1]
Fusarium prolieratum	Fusarium solani
Fusarium subglutinans	Fusarium tricinctum[1]
Fusarium venenatum[1]	Fusarium verticillioides
Hop latent viroid (HLVd)	Lettuce chlorosis virus (LCV)[1]
Monilinia fructicola[1]	Mycosphaerella[1]
Penicillium[1]	Phytophthora cactorum[1]
Phytophthora capsici	Phytophthora ramorum
Puccinia striiformis[1]	Pythium irregulare
Rhizoctonia solani	Sclerotinia[1]
Septoria[1]	Tobacco mosaic virus (TMV)[1]
Verticillium dahliae	
[1]Predicted non-detection by *in silico* analysis only	

Diagnostic Specificity

True Negatives	77
Correct Diagnoses	77
Percent	100%

Selectivity:

No Matrix Effect Observed With:

Alfalfa crowns	Alfalfa roots	Alfalfa seeds	Almond leaves
Apple leaves	Banana corms	Banana leaves	Banana roots
Chrysanthemum leaves	Citrus spp. roots	Citrus spp. stems	Corn leaves
Cotton crown	Cotton leaves	Cotton petioles	Cotton roots
Cotton seeds	Cotton stems	Cucumber leaves	Dahlia leaves
Geranium leaves	Hemp crown	Hemp leaves	Hemp petioles

No Matrix Effect Observed With:

Hemp roots	Hemp stems	Hop leaves	Lettuce crowns
Lettuce leaves	Lettuce roots	Lettuce seeds[1]	Melon leaves
Onion crowns	Onion roots	Onion tubers	Pea crowns
Pea roots	Pea seeds	Pelargonium leaves	Pepper crowns
Pepper seeds	Pistachio leaves	Potato crowns	Potato roots
Potato tubers	Soil	Soybean crowns	Soybean roots
Soybean seeds	Squash leaves	Strawberry crowns	Strawberry roots
Tobacco crowns	Tobacco roots	Tobacco seeds	Tomato crowns
Tomato leaves	Tomato roots	Tomato seeds	Watermelon crowns
Watermelon roots	Watermelon seeds	Well Water	Wheat leaves
[1]False negative observed in 1 out of 2 samples			

Matrix Effect Observed With:

Pepper roots			

Repeatability

Number of Samples	230
Replicates per Sample	2 - 3
Average Percent Agreement Between Replicates	97.4%

Reproducibility

Number of Samples	28
Replicates per Sample	2 - 3
Number of Operators	2
Average Percent Agreement Between Replicates Between Operators	92.4%

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
Positive Sample (Low)	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.

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