Primerdesign™ Ltd

Fusarium_spp

Translation elongation factor alpha (EF-1a) gene

genesig® Standard Kit

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Introduction to Fusarium_spp

The genus Fusarium collectively represents an important group of fungal plant pathogens, causing various diseases on nearly every economically important plant species. Of equal concern is the health hazard posed to humans and livestock by the plethora of Fusarium mycotoxins. Besides their economic importance, species of Fusarium also serve as key model organisms for biological and evolutionary research.

The genus includes a number of economically important plant pathogenic species. Genome size varies amongst species; e.g. FV3 ~41.7 Mbp, FG3 ~36Mbp, FO ~60Mbp. Our kit targets the Translation elongation factor alpha (EF-1a) gene across all Fusarium species.

Members of the Fusarium oxysporum are the most common phytopathogenic Fusaria. They cause wilts of over 100 cultivated plant species, including tomato, potato, sugarcane, bean, cowpea, date and oil palm, as well as cooking and dessert bananas. The primary solution to control such diseases is through the development of disease resistant plant cultivars.

Plant disease resistance genes have been identified for the effective control of tomato wilt but new races of the pathogen continue to develop overcoming deployed resistance and thwarting tomato breeding efforts. Because Fusarium is a long-lived, soil-borne pathogen, infested soil remains contaminated indefinitely, so only resistant varieties can be grown on that site.

Fusarium graminearum commonly infects barley if there is rain late in the season. It is of economic impact to the malting and brewing industries, as well as feed barley. Fusarium contamination in barley can result in head blight, and in extreme contaminations, the barley can appear pink. F. graminearum can also cause root rot and seedling blight.

F. verticillioides is a pathogen of maize and sorghum, and produces carcinogenic mycotoxins known as fumonisins.

Some species may cause a range of opportunistic infections in humans. In humans with normal immune systems, fusarial infections may occur in the nails and in the cornea. In humans whose immune systems are weakened via neutropenia aggressive infections penetrating the entire body and bloodstream may be caused by members of the Fusarium solani complex, Fusarium oxysporum, Fusarium verticillioides, Fusarium proliferatum and, rarely, other fusarial species.

Specificity

The Primerdesign genesig Kit for Fusarium_spp (Fusarium_spp) genomes is designed for the in vitro quantification of Fusarium_spp genomes. The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology with over 95% of the NCBI database reference sequences available at the time of design.

The dynamics of genetic variation means that new sequence information may become available after the initial design. Primerdesign periodically reviews the detection profiles of our kits and when required releases new versions.

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This kit also detects other species including, and not limited to: Ilyonectria, Cylindrodendrum, Neonectria, Neocoasmospora, Nectria, Campylocarpon, Cyanonectria, Xenoacremonium, Cylindrocarpon, Escovopsis, Microcera, Dactylonectria, Phaeoacremonium, Trichoderma, Pestalotiopsis, Purpureocillium, Neurospora.

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to enquiry@primerdesign.co.uk and our bioinformatics

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Kit contents

- **Fusarium_spp specific primer/probe mix (150 reactions BROWN)**
  FAM labelled
- **Fusarium_spp positive control template (for Standard curve RED)**
- **RNase/DNase free water (WHITE)**
  for resuspension of primer/probe mixes
- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template and standard curve preparation

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Extraction kit

This kit is recommended for use with genesig Easy DNA/RNA extraction kit. However, it is designed to work well with all processes that yield high quality RNA and DNA with minimal PCR inhibitors.

oasig™ lyophilised or Precision®PLUS 2X qPCR Master Mix

This kit is intended for use with oasig or PrecisionPLUS 2X qPCR Master Mix.

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and DNA integrity. Always run at least one negative control with the samples. To prepare a negative-control, replace the template DNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions genesig Fusarium_spp detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers

Trademarks

Principles of the test

Real-time PCR

A Fusarium_spp specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the Fusarium_spp DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of Fusarium_spp copy number / Cq value. Alternatively the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target Fusarium_spp gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run.

Fusarium_spp DNA is known to be highly prevalent within the air and environment generally and the negative control may therefore give a late positive signal due to environmental contamination. The interpretation of results section of this handbook gives guidance on how to interpret results where environmental contamination is evident.

Resuspension protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Resuspend the kit components in the RNase/DNase free water supplied, according to the table below.**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in water	Volume
Pre-PCR pack	
Fusarium_spp primer/probe mix (BROWN)	165 µl

3. **Resuspend the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
Fusarium_spp Positive Control Template (RED) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

qPCR detection protocol

1. **For each DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
oasig or PrecisionPLUS 2X qPCR Master Mix	10 µl
Fusarium_spp primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Prepare DNA templates for each of your samples.**

4. **Pipette 5µl of DNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

5. **If a standard curve is included for quantitative analysis, prepare a reaction mix according to the table below:**

Component	Volume
oasig or PrecisionPLUS 2X qPCR Master Mix	10 µl
Fusarium_spp primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

6. Preparation of a standard curve dilution series.

1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
2) Pipette 10µl of Positive Control Template (RED) into tube 2
3) Vortex thoroughly
4) Change pipette tip and pipette 10µl from tube 2 into tube 3
5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

Standard Curve	Copy Number
Tube 1 Positive control (RED)	2 x 10^5 per µl
Tube 2	2 x 10^4 per µl
Tube 3	2 x 10^3 per µl
Tube 4	2 x 10^2 per µl
Tube 5	20 per µl
Tube 6	2 per µl

7. Pipette 5µl of standard template into each well for the standard curve according to your experimental plate set up.
The final volume in each well is 20µl.

qPCR amplification protocol

Amplification conditions using oasig or PrecisionPLUS 2X qPCR Master Mix.

	Step	Time	Temp
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

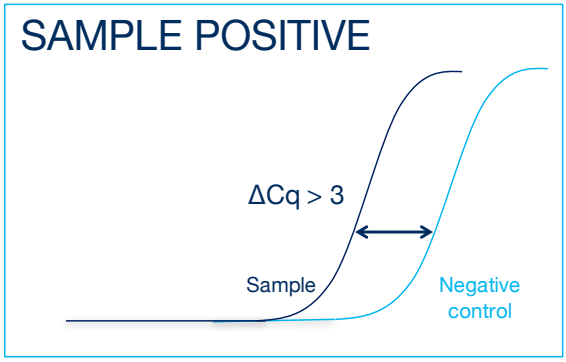
* Fluorogenic data should be collected during this step through the FAM channel

Interpretation of results

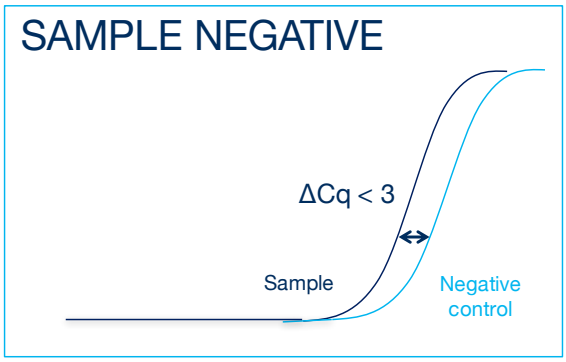
Target	Positive control	Negative control	Interpretation
+	+	-	**POSITIVE QUANTITATIVE RESULT calculate copy number**
-	+	-	**NEGATIVE RESULT**
+ / -	+	≤ 35	**EXPERIMENT FAILED** due to test contamination
+ / -	+	> 35	*
+ / -	-	+ / -	**EXPERIMENT FAILED**

Positive control template (RED) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 3 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 3 Cq earlier than the negative control then the positive sample result is invalidated and a negative call is the correct result.