ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Femto™ Fungal DNA Quantification Kit

Catalog No. E2007

Highlights

- Accurately and reproducibly quantify as little as 20 fg of fungal DNA from 1 µl of sample using real-time PCR.
- High specificity and sensitivity for fungal DNA allows reliable quantification in a background of non-fungal DNA.

Contents

ZYMO RESEARCH CORP.
Phone: (949) 679-1190 • Toll Free: (888) 882-968 • Fax: (949) 266-9452 • www.zymoresearch.com • info@zymoresearch.com

Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product, please call 1-888-882-9682.

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Note - Use of SYTO® 9 is provided under an agreement between Life Technologies Corporation (Molecular Probes Labeling and Detection Technologies) and Zymo Research Corporation and the manufacture, use, sale or import of this product is subject to one or more U.S. Patents and corresponding international equivalents. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation (Molecular Probes Labeling and Detection Technologies), Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300, Fax: (541) 335-0354.

For **Technical Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com

Product Contents:

Femto™ Fungal DNA Quantification Kit	**E2007** 100 rxns.	**Storage Temperature**
Femto™ Fungal qPCR Premix*	1 tube x 1.8 ml	-20 ˚C
Fungal DNA Standards and **No Template Control (#1-7)**	8 tubes x 50 µl	-20 ˚C
Instruction Manual	1	–

Note- Integrity of kit components is guaranteed for one year from date of purchase if proper storage conditions are followed. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

* Femto™ Fungal qPCR Premix includes a fluorescent dye (SYTO® 9) for real-time and quantitative PCR applications.

Specifications:

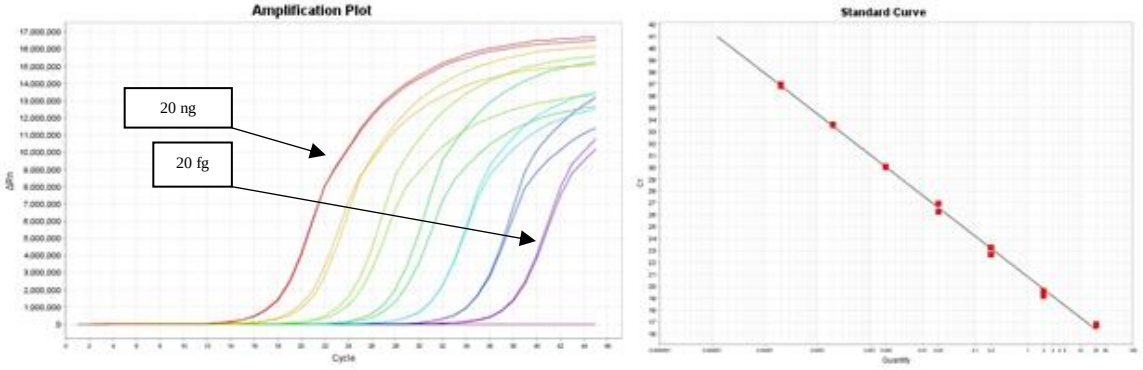
- **Fungal DNA Detection and Quantification:** Detection range of 20 fg-20 ng from as little as 1 µl of sample. The kit can be used to detect ≥2 copies of *Saccharomyces cerevisiae* genomic DNA.
- **Sample source:** Detect and quantify high quality fungal DNA from any purified mixed DNA sample.
- **Compatibility:** Product is designed to be compatible with any real-time and quantitative PCR instrument.

Required equipment and materials not provided in kit:

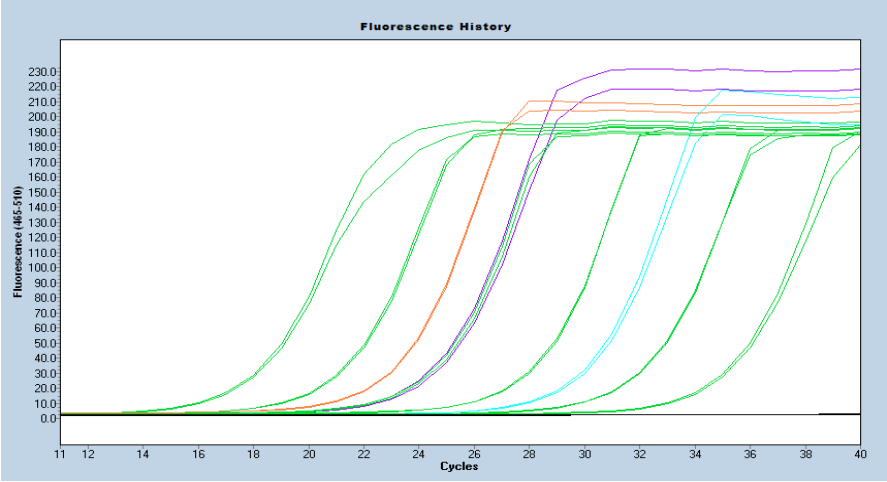
- Real-time quantitative PCR system
- Vortex mixer
- Microcentrifuge
- Pipettes
- Pipette filter tips
- PCR Tube Strip or PCR Plate
- Optically transparent sealing film for PCR plate or tube strip caps

Product Description:

The **Femto™ Fungal DNA Quantification Kit** can be used to detect and quantify fungal DNA with high specificity and sensitivity. Fungal DNA can be reliably quantified in a background of non-fungal DNA such as bacterial, animal, and plant DNA, etc. This is essential for downstream applications that require accurate fungal DNA input including Next-Gen sequencing and metagenomic analysis. With the **Femto™ Fungal DNA Quantification Kit**, one can dependably quantify as little as 20 fg of fungal DNA in 1 µl purified biological liquids, fungal cultures, or environmental DNA samples.



Reliable standards for the quantification of fungal DNA: Fungal DNA Standards (measured in duplicates) comprise 10-fold dilution series ranging from 2 ng to 20 fg.



Amplification of fungal DNA from a variety of samples: Amplification plots of the Femto™ Fungal DNA Quantification Kit of inputs of purified DNA extracted from different sources are shown: Chinchilla feces (purple), sludge (light blue), and soil (orange). Fungal DNA Standards (green) and No Template Control (black) are also shown.

Notes:

[1] It is recommended that the work area for PCR set up is cleaned with a 7% diluted bleach solution to prevent false-positive results.

[2] A vortex mixer is recommended for the thorough mixing of Fungal DNA Standards. If a vortex mixer is not available, carefully mix by pipetting reagent up and down at least 5 times.

Note:
If a concentration greater than 1 ng/µl of DNA is observed, it is recommended to take a small aliquot of sample and dilute 100-fold for accurate quantification.

[3] It is recommended that standards, no template controls, and samples are set up in at least duplicates. See Appendix A on page 4 for an example on PCR plate set up.

Reagent Preparation:

✓ *It is recommended that all reagents and qPCRs be prepared using clean techniques to prevent contamination.* [1]

✓ ***Femto™ Fungal qPCR Premix*** *should be completely thawed at room temperature, mixed by flicking the tube, centrifuged briefly, and then placed on ice. DO NOT VORTEX the Femto™ Fungal qPCR Premix.*

✓ ***Femto™ Fungal qPCR Premix*** *should be protected from direct light exposure. Minimize freeze-thaw cycles.*

✓ ***Fungal DNA Standards (#1-7)*** *should be completely thawed at room temperature, mixed by vortexing, centrifuged briefly, and then placed on ice.* [2]

✓ *All reagents should be kept on ice immediately after thawing.*

Protocol for Fungal DNA Quantification:

Aliquoting Femto™ Fungal qPCR Premix and qPCR setup (*for qPCR tube strips or qPCR plates)*

1. Aliquot 18 µl of the **Femto™ Fungal qPCR Premix** into each well planned for use. [3]
2. Add 2 µl of **Fungal DNA Standards (#1-7)** into the appropriate wells. Remember to change pipette tips after the addition of each Fungal DNA Standard to a well.
3. Add 1 to 3 µl of each Unknown Test Sample to the appropriate wells containing the Master Mix. Remember to change pipette tips after the addition of each Unknown Test Sample. Note: DO NOT ADD Unknown Test Samples to wells containing Fungal DNA Standards or No Template Control.
4. Add 2 µl of the **No Template Control (#8)** into the appropriate wells. Remember to change pipette tips after addition of each No Template Control volume.
5. Seal the qPCR plate with an optically transparent sealing film or qPCR tube strips with tube strip caps that are compatible with the real-time/quantitative PCR instrument being used.
6. Centrifuge the qPCR plate or qPCR tube strips to eliminate bubbles and to bring any droplets to the bottom of the well.

Proceed to the next page for cycling conditions

Thermocycling Parameters:

	Temperature	Time	
-Initial Denaturation	95 ˚C	10 minutes	
-Denaturation	95 ˚C	30 seconds	45 cycles [4]
-Annealing	50 ˚C	30 seconds	
-Extension	72 ˚C	1 minute	
-Final Extension [5]	72 ˚C	7 minutes	

Analysis:

Use the Fungal DNA Standards table below to generate a standard curve to quantify Unknown Test Samples. For example, the Standard 1 wells contain 20 ng of fungal DNA, Standard 2 wells contain 2 ng of fungal DNA, etc.

Fungal DNA Standards	Amount of Fungal DNA Input (ng)/ Reaction Well
Standard 1	20
Standard 2	2
Standard 3	0.2
Standard 4	0.02
Standard 5	0.002
Standard 6	0.0002
Standard 7	0.00002

Appendix A: Sample qPCR Plate Set Up

It is recommended to set up samples (including Fungal DNA Standards and No Template Controls) in at least duplicates.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1										
B	Std 2	Std 2										
C	Std 3	Std 3										
D	Std 4	Std 4										
E	Std 5	Std 5										
F	Std 6	Std 6										
G	Std 7	Std 7										
H	NTC	NTC										

Unknown Test Samples

Example of Plate Setup: 96-well PCR plate set up for Fungal DNA Standards (Std) and No Template Control (NTC). All other empty wells may be used for Unknown Test Sample input.

[4] The number of cycles can be adjusted if desired, but a minimum of 45 cycles is required to completely resolve the standard curve.

[5] If desired, an additional dissociation analysis (melting curves) step may be added after the final extension step is completed.

Note:
Please refer to the instrument's manual for more detailed instructions on setting up the standard curve.

Appendix B: Fungal Primers and Fungal DNA Standards

This kit contains a primer mix targeting the ITS region. The Fungal DNA Standards provided are purified from S. cerevisiae strain TMY18. The genomic copy number of the ITS region may vary between fungal species. This kit provides an exact quantity of fungal DNA present and gives an approximate number of fungal cells present based on S. cerevisiae strain TMY18 copy number. [6]

[6] Dubouzet J, Shinoda K (1999) Relationships among Old and New World Alliums according to ITS DNA sequence analysis. Theoretical and Applied Genetics doi: 10.1007/s001220051088

Appendix C: Troubleshooting

No Amplification of Fungal DNA Standards or Unknown Test Samples

- **One or more qPCRs components may be missing, or Fungal DNA Standards or Unknown Test Samples may not have been added.**

  ✓ Repeat the qPCR experiment, making sure that Femto™ Fungal qPCR Premix, Fungal DNA Standards, and/or Unknown Test Samples are added according to the protocol. Be sure to add Fungal DNA Standards or Unknown Test Samples to the appropriate wells directly. Avoid pipetting onto well walls.

- **High amounts of PCR Inhibitors (excess salts such as NaCl and KCl, ethanol, isopropanol, polyphenolics, humic acid, guanidinium, ionic detergents such as SDS and sarkosyl, etc.).**

  ✓ PCR inhibitors may hinder the enzymatic reactions of DNA polymerase. Ensure that the method of sample collection effectively excludes PCR inhibitors, and purify samples if needed. [7]

[7] **OneStep™ PCR Inhibitor Removal Kit** removes enzymatic inhibitors including polyphenolics, humic/fulvic acids, tannins, melanin, etc. from impure DNA preparation.. (Cat. No. D6030)

DNA Clean & Concentrator™-5 facilitates the rapid purification and concentration of high-quality DNA from endonuclease digestions, cell lysates, and other impure DNA preparations. (Cat No. D4003)

High Standard Deviation in Fungal DNA Standard or Unknown Test Sample Replicate Groups

- **Reaction volumes are inconsistent between wells.**

  ✓ Make sure to add Femto™ Fungal qPCR Premix as well as Fungal DNA Standards or Unknown Test Samples directly to the well and not the sides of the wells. Remember to centrifuge the qPCR plate or qPCR tube strips to bring any droplets to the bottom of the wells.

  ✓ Pipetting volumes may not be accurate, be sure all pipettes are calibrated. PCR plate or PCR tube strips should be sealed properly in order to prevent any evaporation or condensation of reagents.

Amplification of No Template Control with less than 38 cycles

- **Introduction of contamination during aliquotting the Femto™ Fungal qPCR Premix, Fungal DNA Standards, or Unknown Test Samples.**

  ✓ Decontaminate pipettes/work area with a 7% diluted bleach solution. Use pipette filter tips when aliquoting Femto™ Fungal qPCR Premix. Make sure to use caution when pipetting Femto™ Fungal qPCR Premix into appropriate wells. Ensure that no Unknown Test Samples or Fungal DNA Standards are added to the No Template Control wells. Use clean procedures to prevent introduction of contamination.

Note: The high sensitivity of this assay mandates that clean techniques are used.

Ordering Information:

Product Description	Catalog No.	Kit Size
Femto™ Fungal DNA Quantification Kit	E2007	100 rxns.

For Individual Sale	Catalog No.	Amount(s)
Femto™ Fungal qPCR Premix	E2007-1	1.8 ml
Fungal DNA Standards and No Template Control (#1-8)	E2007-2	50 µl x 8 tubes