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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 μ l of sample using real-time PCR.
- High specificity and sensitivity for fungal DNA allows reliable quantification in a background of non-fungal DNA.

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Note - TM 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.

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For **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.

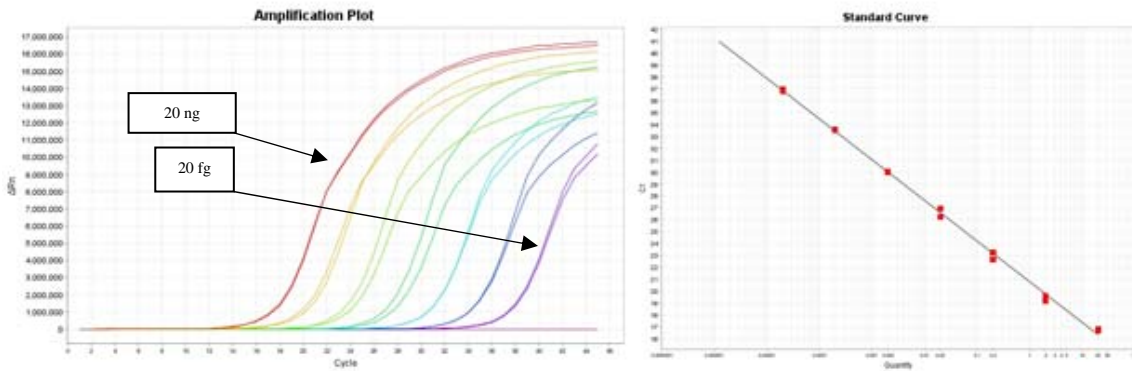
Required equipment and materials not provided in kit:

- 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

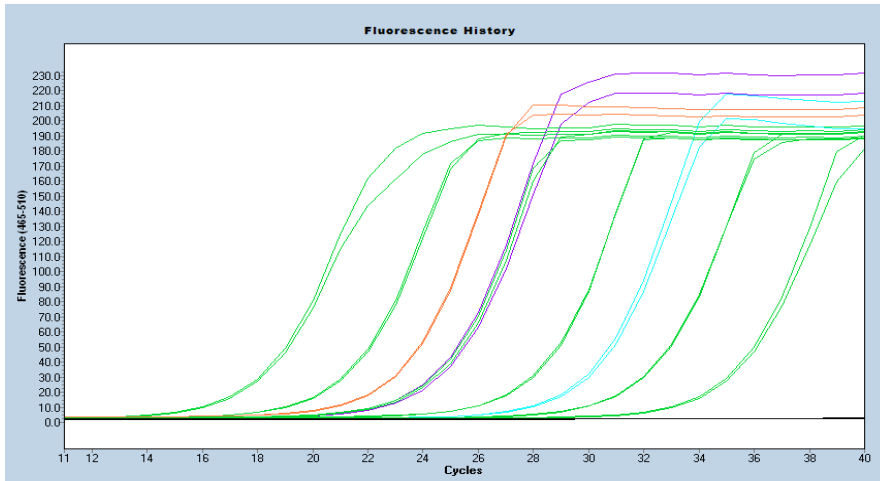
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Product Description:

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 μ l 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.

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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:

	<u>Temperature</u>	<u>Time</u>	
-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	

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.

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

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

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.

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

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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)

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

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

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.

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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

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Xpedition™ Sample Prep & Preservation Technologies: The Complete Solution to “TAKE THE LAB TO THE FIELD”

Workflow (e.g., DNA Purification)



Lyse/stabilize samples during your expedition using Xpedition™ Sample Processor & Lysis/Stabilization Solution

Store/transport lysates without refrigeration

Process *later* using Xpedition™ DNA Prep Kits

Intact, inhibitor-free DNA ready for analysis!

For Portable Sample Lysis...








Xpedition™ Sample Processor

S6020	Xpedition™ Sample Processor	1 unit
D6202	Xpedition™ Soil/Fecal DNA MiniPrep	50 preps.
D6206	Xpedition™ Fungal/Bacterial DNA MiniPrep	50 preps.
D6216	Xpedition™ Tissue & Insect DNA MiniPrep	50 preps.
D6221	Xpedition™ Plant/Seed DNA MiniPrep	50 preps.
D6202-1-40	Xpedition™ Lysis/Stabilization Solution	40 ml





All Xpedition™ DNA MiniPrep Kits feature a unique lysis/stabilization solution. This allows high quality DNA to be isolated immediately or when convenient for the researcher following storage/transport of sample lysates.

Other Popular Environmental DNA/RNA Purification Products

ENVIRONMENTAL DNA

	D6003	ZR Soil Microbe DNA MicroPrep™	50 preps.
	D6001	ZR Soil Microbe DNA MiniPrep™	50 preps.
	D6101	ZR Soil Microbe DNA MidiPrep™	25 preps.
	D6002	ZR-96 Soil Microbe DNA Kit™	2 x 96 preps.
	D6007	ZR Fungal/Bacterial DNA MicroPrep™	50 preps.
	D6005	ZR Fungal/Bacterial DNA MiniPrep™	50 preps.
	D6105	ZR Fungal/Bacterial DNA MidiPrep™	25 preps.
	D6006	ZR-96 Fungal/Bacterial DNA Kit™	2 x 96 preps.
	D6012	ZR Fecal DNA MicroPrep™	50 preps.
	D6010	ZR Fecal DNA MiniPrep™	50 preps.
	D6110	ZR Fecal DNA MidiPrep™	25 preps.
	D6011	ZR-96 Fecal DNA Kit™	2 x 96 preps.
	D6015	ZR Tissue & Insect DNA MicroPrep™	50 preps.
	D6016	ZR Tissue & Insect DNA MiniPrep™	50 preps.
	D6115	ZR Tissue & Insect DNA MidiPrep™	25 preps.
	D6017	ZR-96 Tissue & Insect DNA Kit™	2 x 96 preps.
	D6022	ZR Plant/Seed DNA MicroPrep™	50 preps.
	D6020	ZR Plant/Seed DNA MiniPrep™	50 preps.
	D6120	ZR Plant/Seed DNA MidiPrep™	25 preps.
	D6021	ZR-96 Plant/Seed DNA Kit™	2 x 96 preps.

ENVIRONMENTAL RNA

	R2040	ZR Soil/Fecal RNA MicroPrep™	50 preps.
	R2010	ZR Fungal/Bacterial RNA MicroPrep™	50 preps.
	R2014	ZR Fungal/Bacterial RNA MiniPrep™	50 preps.
	R2030	ZR Tissue & Insect RNA MicroPrep™	50 preps.
	R2024	ZR Plant RNA MiniPrep™	50 preps.

ALSO AVAILABLE

D6030	OneStep™ PCR Inhibitor Removal Kit	50 preps.
D6035	OneStep-96™ PCR Inhibitor Removal Kit	2 x 96 preps.
S6002-50	ZR BashingBead™ Lysis Tubes (0.5 mm)	50 tubes
S6003-50	ZR BashingBead™ Lysis Tubes (2.0 mm)	50 tubes
S6002-96-1	ZR-96 BashingBead™ Lysis Rack (0.5 mm)	1 rack
S6002-96-2	ZR-96 BashingBead™ Lysis Rack (2.0 mm)	1 rack



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See the Xpedition™ Sample Processor in action!

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