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- Trockeneiszuschlag
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ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Femto™ Bacterial DNA Quantification Kit

Catalog No. E2006

Highlights

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

Contents

ZYMO RESEARCH CORP.

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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.

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Product Contents:

Femto™ Bacterial DNA Quantification Kit	E2006 100 rxns.	Storage Temperature
Femto™ Bacterial qPCR Premix*	1 tube x 1.8 ml	-20 ˚C
Bacterial DNA Standards and **No Template Control (#1-8)**	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™ Bacterial qPCR Premix includes a fluorescent dye (SYTO® 9) for real-time and quantitative PCR applications.

Specifications:

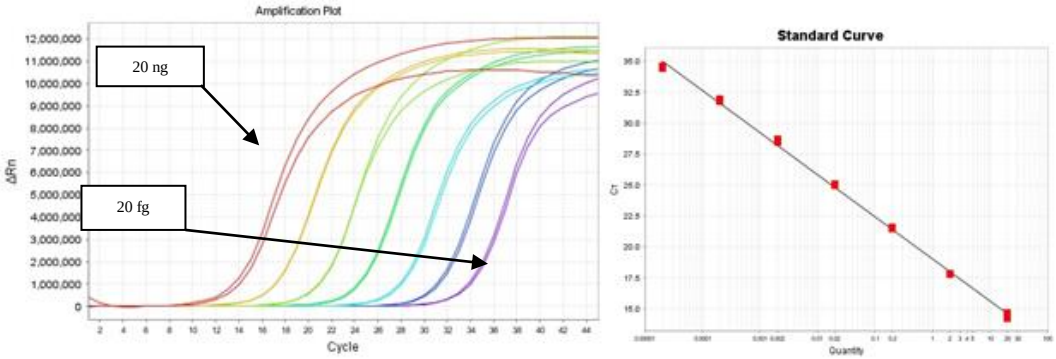
- **Bacterial DNA Detection and Quantification:** Detection range of 20 fg-20 ng from as little as 1 µl of sample. The kit can be used to detect down to 5 copies of Escherichia coli genomic DNA.
- **Sample source:** Detect and quantify high quality bacterial DNA from any purified mixed DNA sample.
- **Compatibility:** Product is designed to be compatible with any real-time and quantitative PCR instrument.

Required equipment and materials not provided in kit:

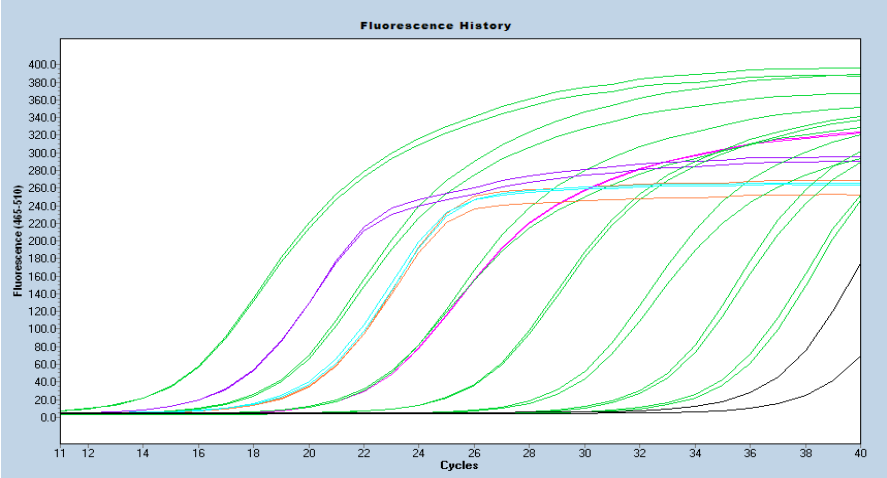
- Real-time quantitative PCR system
- Vortex mixer
- Microcentrifuge
- Pipettes
- Pipette filter tips
- PCR Tube Strip or PCR Plate
- Optically transparent sealing film for PCR plate or tube strip caps

Product Description:

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



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



Amplification of bacterial DNA from a variety of samples: Amplification plots of the Femto™ Bacterial DNA Quantification Kit of inputs of purified DNA extracted from different sources are shown: Chinchilla feces (purple), sludge (light blue), soil (orange), and E. coli culture (pink). Bacterial 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 Bacterial 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 10 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™ Bacterial qPCR Premix*** *should be completely thawed at room temperature, mixed by flicking the tube, centrifuged briefly, and then placed on ice. DO NOT VORTEX Femto™ Bacterial qPCR Premix.*

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

✓ ***Bacterial 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 Bacterial DNA Quantification:

Aliquoting Femto™ Bacterial qPCR Premix and qPCR setup (for q*PCR tube strips or qPCR plates*)

1. Aliquot 18 µl of the **Femto™ Bacterial qPCR Premix** into each well planned for use. [3]
2. Add 2 µl of **Bacterial DNA Standards (#1-7)** into the appropriate wells. Remember to change pipette tips after the addition of each Bacterial 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 Bacterial 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 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	40 cycles [4]
-Annealing	50 ˚C	30 seconds	
-Extension	72 ˚C	1 minute	
-Final Extension [5]	72 ˚C	7 minutes	

Analysis:

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

Bacterial DNA Standards	Amount of Bacterial 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 all samples (including Bacterial 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 Set Up: 96-well PCR plate set up for Bacterial 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 36 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: Bacterial Primers and Bacterial DNA Standards

This kit contains a primer mix targeting the 16S rRNA. The Bacterial DNA Standards provided are purified from E. coli strain JM109. The genomic copy number of the 16S rRNA may vary between bacterial species. This kit provides an exact quantity of bacterial DNA present and gives an approximate number of bacterial cells based on E. coli strain JM109 copy number. [6]

Appendix C: Troubleshooting

No Amplification of Bacterial DNA Standards or Unknown Test Samples

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

  ✓ Repeat the qPCR experiment, making sure that Femto™ Bacterial qPCR Premix, Bacterial DNA Standards, and/or Unknown Test Samples are added according to the protocol. Be sure to add Bacterial 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 Bacterial DNA Standard or Unknown Test Sample Replicate Groups

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

  ✓ Make sure to add Femto™ Bacterial qPCR Premix as well as Bacterial 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 33 cycles

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

  ✓ Decontaminate pipettes/work area with a 7% diluted bleach solution. Use pipette filter tips when aliquoting Femto™ Bacterial qPCR Premix. Make sure to use caution when pipetting Femto™ Bacterial qPCR Premix into appropriate wells. Ensure that no Unknown Test Samples or Bacterial 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.

[6] Větrovský T, Baldrian P (2013) The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses. PLoS ONE 8(2): e57923. doi: 10.1371/journal.pone.0057923

[7] **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)

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

Ordering Information:

Product Description	Catalog No.	Kit Size
Femto™ Bacterial DNA Quantification Kit	E2006	100 rxns.

For Individual Sale	Catalog No.	Amount(s)
Femto™ Bacterial qPCR Premix	E2006-1	1.8 ml
Bacterial DNA Standards and No Template Control (#1-8)	E2006-2	50 µl x 8 tubes