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INSTRUCTION MANUAL

Femto™ Human DNA Quantification Kit

Catalog No. E2005

Highlights

- Accurately and reproducibly quantify as little as 20 fg of human DNA using real-time PCR.
- High specificity and sensitivity for human DNA allows for reliable quantification in a background of non-human 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 **Technical Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com

Product Contents:

Femto[™] Human DNA Quantification Kit	E2005 100 rxns.	Storage Temperature
Femto[™] Human qPCR Premix*	1 tube x 1.8 ml	-20 °C
Human 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[™] Human qPCR Premix includes a fluorescent dye (SYTO[®] 9) for real-time and quantitative PCR applications.

Specifications:

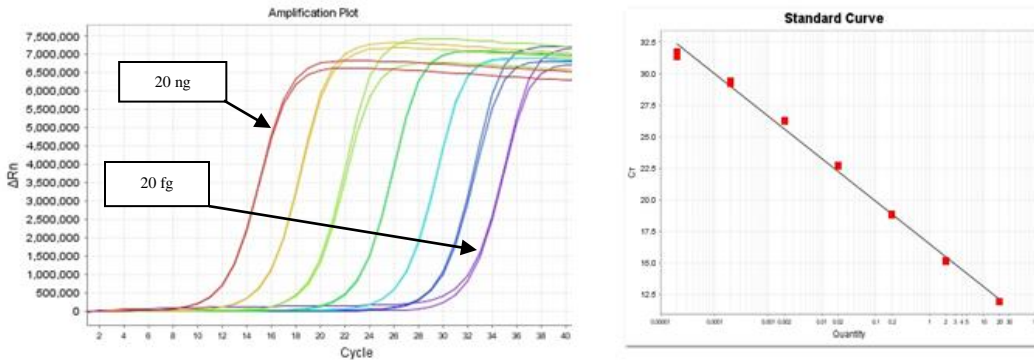
- **Human DNA Detection and Quantification:** Detection range of 20 fg-20 ng from as little as 1 µl of sample. The kit can be used to detect less than 1 copy of human gDNA.
- **Sample source:** Detect and quantify high quality human DNA from any purified mixed DNA sample.
- **Compatibility:** Product is designed to be compatible with any real-time and quantitative PCR instrument.

Required equipment and materials not provided in kit:

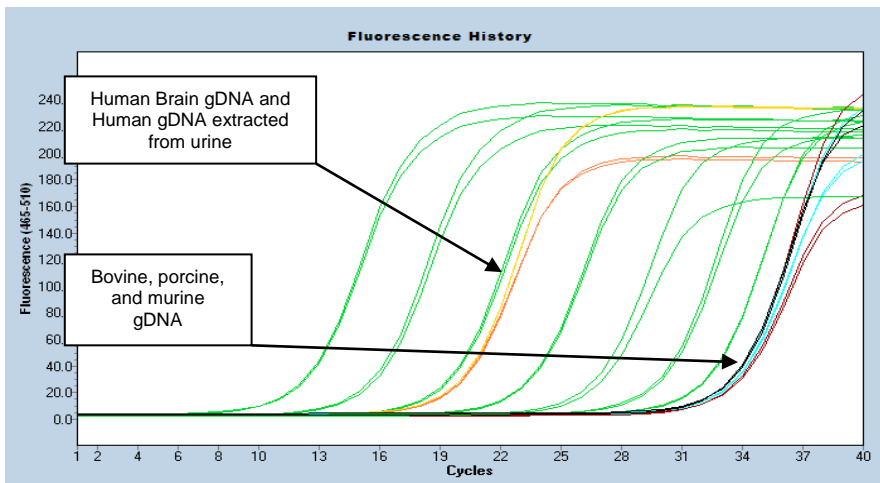
- Real-time quantitative PCR system
- Vortex mixer
- Microcentrifuge
- Pipettes
- Pipette filter tips
- PCR Tube Strip or PCR Plate
- Optically transparent sealing film for PCR plate or tube strip caps

Product Description:

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



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



Specificity: Amplification plots demonstrate the specificity of the Femto™ Human DNA Quantification Kit. Bovine gDNA, porcine gDNA, and murine gDNA exhibited delayed amplification similar to the No Template Control (≥ 35 cycles). 0.2 ng of human brain gDNA (orange), human gDNA extracted from urine (gold), bovine gDNA (red), porcine gDNA (light blue), and murine gDNA (purple) were used.

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 Human 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/ μ l 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 qPCR plate set up.

Reagent Preparation:

- ✓ *It is recommended that all reagents and qPCRs be prepared using clean techniques to prevent contamination.*¹
- ✓ ***Femto™ Human 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™ Human qPCR Premix.*
- ✓ ***Femto™ Human qPCR Premix** should be protected from direct light exposure. Minimize freeze-thaw cycles.*
- ✓ ***Human 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 Human DNA Quantification:

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

1. Aliquot 18 μ l of the **Femto™ Human qPCR Premix** into each well planned for use.³
2. Add 2 μ l of **Human DNA Standards (#1-7)** into the appropriate wells. Remember to change pipette tips after the addition of each Human DNA Standard to a well.
3. Add 1 to 3 μ l 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 Human DNA Standards or No Template Control.
4. Add 2 μ l 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	} 40 cycles ⁴
-Annealing	59 °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 34 cycles is required to completely resolve the standard curve.

Analysis:

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

Human DNA Standards	Amount of Human 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

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 A: Sample qPCR Plate Set Up

It is recommended to set up all samples (including Human 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										

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

Appendix B: Troubleshooting

No Amplification of Human DNA Standards or Unknown Test Samples

- **One or more qPCR components may be missing, or Human DNA Standards or Unknown Test Samples may not have been added.**
 - ✓ Repeat the qPCR experiment, making sure that Femto™ Human qPCR Premix, Human DNA Standards, and/or Unknown Test Samples are added according to the protocol. Be sure to add Human 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.⁶

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

- **Reaction volumes are inconsistent between wells.**
 - ✓ Make sure to add Femto™ Human qPCR Premix as well as Human 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 30 cycles

- **Introduction of contamination during aliquotting the Femto™ Human qPCR Premix, Human DNA Standards, or Unknown Test Samples.**
 - ✓ Decontaminate pipettes/work area with a 7% diluted bleach solution. Use pipette filter tips when aliquoting Femto™ Human qPCR Premix. Make sure to use caution when pipetting Femto™ Human qPCR Premix into appropriate wells. Ensure that no Unknown Test Samples or Human 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 **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)

Ordering Information:

Product Description	Catalog No.	Kit Size
Femto™ Human DNA Quantification Kit	E2005	100 rxns.

For Individual Sale	Catalog No.	Amount(s)
Femto™ Human qPCR Premix	E2005-1	1.8 ml
Human DNA Standards and No Template Control (#1-8)	E2005-2	50 µl x 8 tubes