applied biosystems

QuantStudio™ 3D Digital PCR Reagent Kit

Catalog Number A26360

Pub. No. 100027337 **Rev.** A

Contents	Cat. no. A26360			Storage conditions
	Part no.	Quantity	Conc.	
CEPH 1347-02 (human female genomic DNA)	361964	180 µL	50 ng/µL	–20°C (original tube and any aliquots)
GLA Assay, FAM™ Dye (human)	100026432	45 µL	20X	–20°C
RNase P Assay, VIC™ Dye (human)	100026301	45 µL	20X	–20°C
QuantStudio™ 3D Digital PCR Master Mix v2	100027614	1.5 mL	2X	–20°C until first use, then 2 to 8°C
Nuclease-free Water	4469813	1.75 mL	—	Room temperature

Note: For safety and biohazard guidelines, refer to the "Safety" appendix in the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The QuantStudio™ 3D Digital PCR Reagent Kit includes genomic DNA, assays, and reagents that enable you to confirm the performance of the QuantStudio™ 3D Digital PCR System either at the time of system installation or as part of future system verification.

Additional required materials

In addition to the QuantStudio™ 3D Digital PCR Reagent Kit, the following kit is required to complete the procedure in this information sheet. Both kits are included as part of the QuantStudio® 3D Digital PCR Starter Kit (Cat. no. A26361). These products can be ordered from **www.lifetechnologies.com**.

Cat. no.	Contents	Quantity
A26316	QuantStudio™ 3D Digital Chip Kit v2	1 kit, containing: • 12 v2 chips, loading blades, and chip lids • 3 syringes of Immersion Fluid, plus tips

Workflow

Start

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Dilute the genomic DNA

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Prepare the digital PCR (dPCR) reaction by mixing genomic DNA, QuantStudio™ 3D Digital PCR Master Mix v2, and assays

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Load the PCR reaction onto a QuantStudio™ 3D Digital PCR Chip v2, apply a lid, fill the assembly with immersion fluid, then seal the loading port

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Thermal cycle the chip

▼

Read the chips using the QuantStudio™ 3D Digital PCR Instrument

▼

Analyze the data using the QuantStudio™ 3D AnalysisSuite™ Software

▼

Finish

Note: Refer to the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720) for performing a single digital PCR (dPCR) experiment on the QuantStudio™ 3D Digital PCR System. Refer to the AnalysisSuite™ Software help system for detailed information on data analysis.

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Determining the ratio of human GLA target gene to human RNase P reference gene

The following protocol uses the QuantStudio™ 3D Digital PCR System and the components of this kit to calculate the ratio of the human GLA target gene to the human RNase P reference gene in a human female genomic DNA sample (CEPH 1347-02). Since each gene should be present at the rate of a single copy per haploid human female genome, the expected ratio between measurements for each assay is 1.0 ± 10%.

Dilute the genomic DNA

The human DNA provided in the kit (CEPH 1347-02) must first be diluted down to a limiting quantity prior to running the dPCR experiment. For this protocol, we recommend diluting the DNA to a final concentration of ≈1,000 copies/µL. In general, we recommend loading approximately 0.6 to 1.6 copies of the target sequence per reaction well in the QuantStudio™ 3D Digital PCR Chip v2, which is in the range of 200 to 2,000 copies/µL of the target sequence in the reaction.

Dilute the DNA from the stock concentration to a working concentration prior to preparing the dPCR reaction mix:

1. Thaw on ice, vortex, then briefly centrifuge the DNA.

   Note: Do not perform more than 10 freeze-thaw cycles. If you expect to freeze-thaw more than three times, we recommend that you aliquot the DNA to minimize the number of freeze-thaw cycles.

2. Using a permanent marker, label a 0.5- or 1.5-mL reaction tube.

3. Prepare the dilution by transferring the appropriate volume of nuclease-free water, then the corresponding volume of DNA to the labeled tube as shown below.

Material	Volume	DNA conc.
Nuclease-free water	8.3 µL	—
CEPH 1347-02 (human female genomic DNA)	16.7 µL	**Stock:** ≈15,000 Copies/µL (or 50 ng/µL)
Total	25 µL	**Working:** 10,000 Copies/µL (or 10X)

4. Cap the tube, vortex, then briefly centrifuge the diluted DNA.

Prepare the dPCR reactions

Use the following procedure to prepare the dPCR reactions for the genomic DNA sample and assays that you will load into the chip. The volumes have been adjusted so that three replicates (chips) are run for the sample and assay combination.

1. Thaw the following at room temperature, and ensure that the tubes are at room temperature before using:
   - QuantStudio™ 3D Digital PCR Master Mix v2
   - GLA Assay, FAM™ Dye
   - RNase P Assay, VIC™ Dye

2. When the master mix is at room temperature, gently invert the tube 10 times.

3. Vortex, then briefly centrifuge the diluted genomic DNA and the assays.

4. In an appropriate reaction tube, combine the dPCR reaction mix components as shown in the following table.

Material	Initial conc.	Final conc.	Volume (µL)	
			Per chip	3 chips [1]
QuantStudio™ 3D Digital PCR Master Mix v2	2X	1X	7.3	26.3
GLA Assay	20X	1X	0.7	2.5
RNase P Assay	20X	1X	0.7	2.5
Diluted genomic DNA	10X	1X	1.4	5.0
Nuclease-free water	—	—	4.4	15.8
Total volume	**—**	**—**	**14.5**	**52.1**

[1] Includes 20% excess volume to account for pipetting errors.

5. Mix well by gently pipetting up and down.

6. Cap the reaction tube, then centrifuge for 1 minute at 1,000 rpm to eliminate any bubbles from the reaction. Proceed immediately to load the QuantStudio™ 3D Digital PCR Chip v2.

IMPORTANT! For optimal results, load the chip as soon as possible after setting up the reactions.

Load and run the reactions

Refer to the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720) for procedures on how to load, thermal cycle, image, and analyze the chips.

Expected analysis results

A calculated GLA assay (FAM™ Dye)/RNase P assay (VIC™ Dye) ratio of 1.0 ± 10% confirms the performance of the QuantStudio™ 3D Digital PCR System. More information on the expected analysis results are shown below.

IMPORTANT! If you do not receive the expected results when using the described protocol, troubleshoot the chip image in the software as described in the user documentation for the QuantStudio™ 3D Digital PCR System and the AnalysisSuite™ Software. If you cannot troubleshoot the results, then contact Technical Support.

Example of expected analysis results

Figure 1 below shows an example of the expected results when using the described protocol. The genomic DNA was diluted to a working concentration as described in this protocol, loaded in a total volume of 14.5 µL to each replicate chip, and amplified using both FAM™ and VIC™ Dye-labeled assays.

The chip in Figure 1 was analyzed using the default quality threshold and 95% confidence level in the QuantStudio™ 3D AnalysisSuite™ Software and is displayed by the assigned call, based on the target (dye) signal detected in each reaction well of the chip.

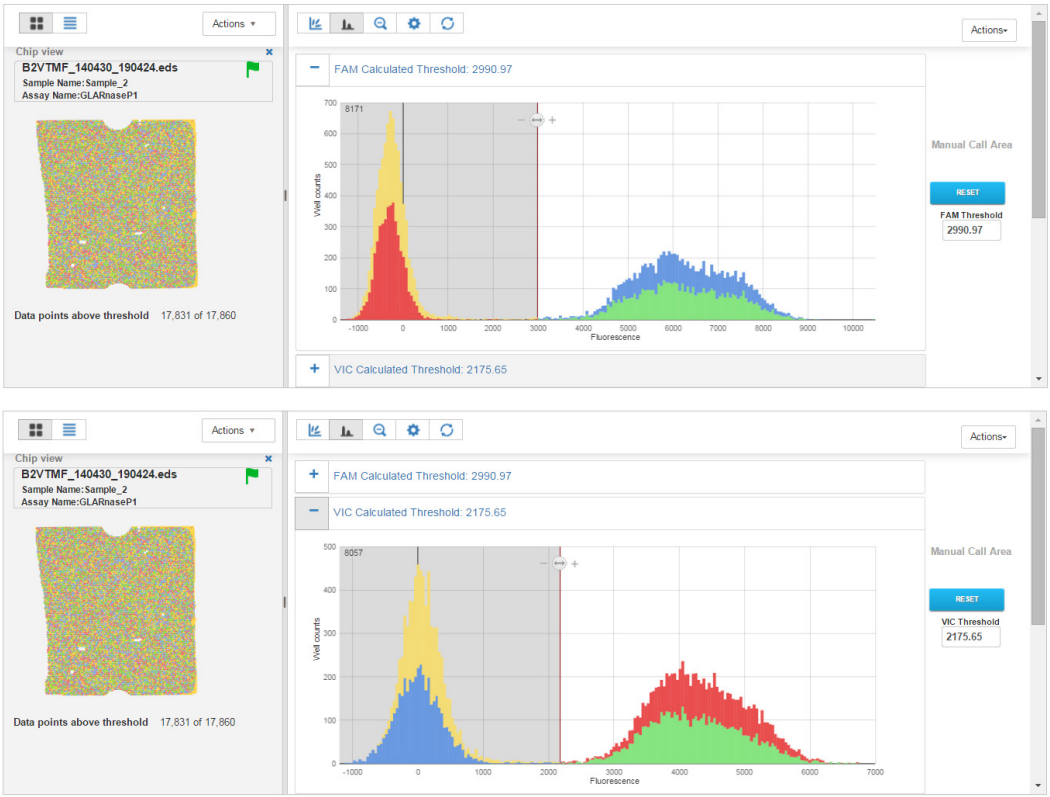

Figure 1 Analysis results in the AnalysisSuite™ Software

Passing criteria

Passing criteria for all replicate chips include:

- A (green) data quality flag in the instrument touchscreen or in the Review Data tab of the AnalysisSuite™ Software

  Note: A (yellow) data quality flag may indicate possible problems with the consumables or workflow that are independent of instrument and reagent performance.

- Uniform spatial distribution of calls across the chip, as shown in the Chip view under the Review Data tab of AnalysisSuite™ Software

  Note: To calculate a quality value for each chip well, the software assesses the distribution of positive and negative calls across the chip and compares the expected clustering of high FAM™ or VIC™ Dye signal *at a given concentration* against the observed clustering. Wells that form groups of positives that cluster differently from what is expected at the estimated concentration are assigned lower quality values. Wells that do not meet the default quality value threshold of 0.5 are automatically excluded from further analysis.

- Bi-modal (two peak) distribution of both FAM™ and VIC™ Dyes with clear separation between the positive and negative peaks, as shown in the Histogram view of AnalysisSuite™ Software

- Assuming there are no significant workflow errors (including DNA concentration, dilution, or pipetting):
  - Copies/µL (C/µL) for both FAM™ and VIC™ Dyes is within ± 10% of the mean concentration
  - GLA assay (FAM™ Dye)/RNase P assay (VIC™ Dye) ratio is within ± 10% of the expected 1.0 ratio

    Note: To calculate the dye ratio, you can use either the C/µL values shown in the instrument touchscreen or the Copies/µL values shown in the Results tab of AnalysisSuite™ Software.

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- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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