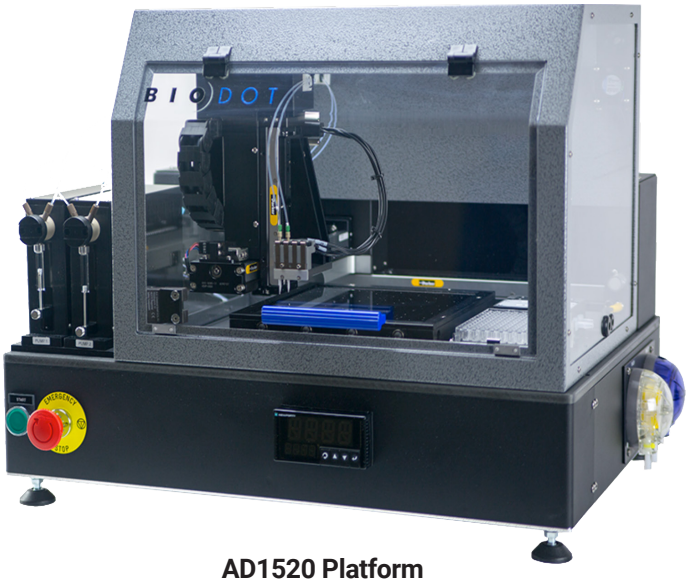
Achieving Nanoliter-Scale PCR with BioDot AD1520™: Reliable, Low-Volume PCR Reactions.



AD1520 Platform

Challenge

Polymerase Chain Reaction (PCR) reagents are among the most expensive consumables in genomics workflows. High-throughput labs must reduce volumes without sacrificing reaction consistency or risking contamination. Droplet-based low-volume PCR offers a promising solution but it requires precise dispensing, thermal control, and validated cleaning protocols.

Solution

The BioDot AD1520 was used to successfully dispense 400 nL PCR reactions into a 384-well plate with a chilled block setup. Each reaction maintains amplification quality comparable to manually pipetted controls. Cross-contamination was mitigated through an effective bleach cleaning step between reagents and templates. This workflow was validated using TaqMan™ reagents and can be adapted with alternative PCR reagent systems.

Materials

AD1520 BioDot liquid dispensing platform.

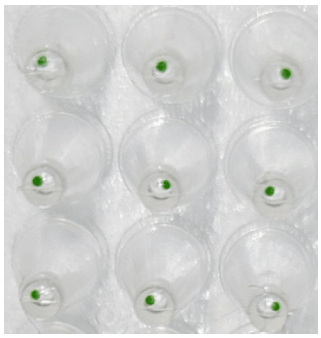
- One BioJet™ dispenser.

Reagents

- Invitrogen RT-PCR Grade Water (Thermo Fisher AM9935).
- TaqMan Universal PCR Master Mix (Thermo Fisher 4304437).
- TaqMan Exogenous Internal Positive Control Reagents (Thermo Fisher 4308321).

Additional Equipment and Consumables

- Falcon 96-well plate Flat Bottom Microplate (Corning 351172).
- MicroAmp Optical 384-well Reaction Plate (Thermo Fisher 4309849).
- MicroAmp Optical Adhesive Film (Thermo Fisher 4311971).
- Swinging Bucket Centrifuge, refrigerated.
- QuantStudio 7 Pro, (Thermo Fisher).



:SIJWXNIJ [NJ\ TK F \JQQ WJFHYNTS UQFGNJXMTSNSL PCR droplets dispensed into a well using a protocol to minimize evaporation. Subsequent centrifugation brings the PCR components together to be loaded into a thermocycler.

Dave Pai
Field Application Scientist

Dave has built his career in customer-facing scientific roles, specializing in training, support, and troubleshooting for genomics and molecular biology platforms. He brings a strong foundation in molecular biology, biochemistry, and genomics. This role at BioDot, where he applies his expertise to optimize low-volume dispensing technologies. His focus is on helping customers improve efficiency and reduce operational costs within their existing workflows.

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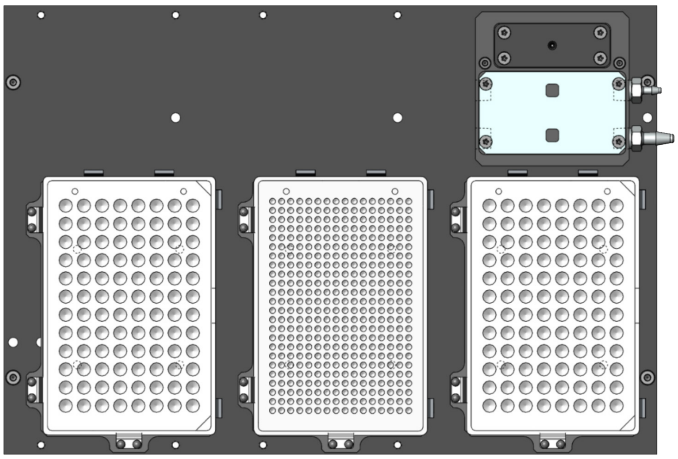


Top-down view of the AD1520 nest showing a 96-well source plate on the left, a 384-well reaction plate in the center, and an additional source plate on the right. Wash, waste, and vacuum stations are located in the top right

Sample Preparation Method

All PCR dispensing was conducted using the BioDot AD1520 equipped with a 190 µm BioJet tip. Nuclease-free water was used throughout the system to prevent contamination.

Each qPCR reaction included three components:

- DNA Template (TaqMan™ Exogenous Internal Positive Control
- Reagents).
- Primer-Probe Mix (TaqMan™ Exogenous Internal Positive Control Reagents).
- Master Mix (TaqMan™ Universal PCR Master Mix).

Each reagent was diluted to create a working solution such that the final concentration in the reaction well was 1X. These working dilutions were loaded into a 96-well flat-bottom "Source Plate" placed at ambient temperature on the dispenser nest.

A MicroAmp™ Optical 384-well Reaction Plate was prepared with a protocol to minimize evaporation prior to dispensing. The Reaction plate was positioned on a chilled aluminum block (15°C) connected to a circulating chiller, with ambient humidity in the AD1520 chamber maintained at 45% to prevent condensation.

Dispensing Parameters

For each well, the BioJet tip aspirated 12 µL of reagent from the Source plate and dispensed the following volumes into the Reaction plate for a total reaction volume of 400 nL through non-contact dispensing:

- 100 nL of a 4X DNA template solution.
- 100 nL of a 4X primer-probe solution.
- 200 nL of a 2X Master Mix solution.

To prevent cross-contamination, a bleach cleaning cycle was performed after dispensing each reagent, to ensure complete removal of that reagent, prior to aspirating the next reagent. The tip is submerged in a solution of 10% bleach, after which the bleach solution is aspirated into the tip and dispensed into a neighboring reservoir. Water is then flushed through the tip, followed by a water rinse of the outside of the tip, and a vacuum dry.

After reagent dispensing, each well was further sealed with a protocol to minimize evaporation. Plates were sealed with MicroAmp™ Optical Adhesive Film and centrifuged at 4,200 × g for 10 minutes at 15°C.

Controls

Positive controls were prepared by manually pipetting 5 µL of the same PCR reaction as above (1.25 µL of the 4X DNA template, 1.25 µL of the 4X primer-probe solution, and 2.5 µL of the 2X Master Mix) into 3 separate wells. A 5 µL manually-pipetted no template control was performed in parallel to ensure that PCR reagents were nuclease-free.

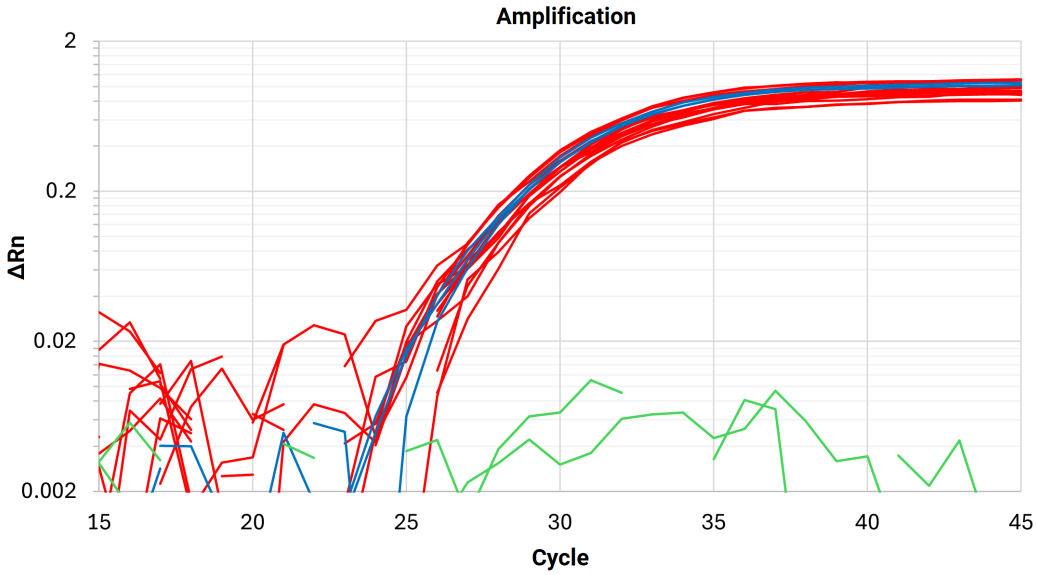
Thermal Cycling and Analysis

qPCR was performed using the QuantStudio™ 7 Pro with the following program:

- 50°C for 2 min.
- 95°C for 10 min.
- 45 cycles: 95°C for 15 sec, 60°C for 1 min.

Detection was performed using VIC as the reporter dye and TAMRA as the quencher, as required by the TaqMan™ Exogenous Internal Positive Control reagents. Data analysis was completed using Thermo Fisher Design and Analysis Software 2 (DA2), Version 2.8.0.

Results



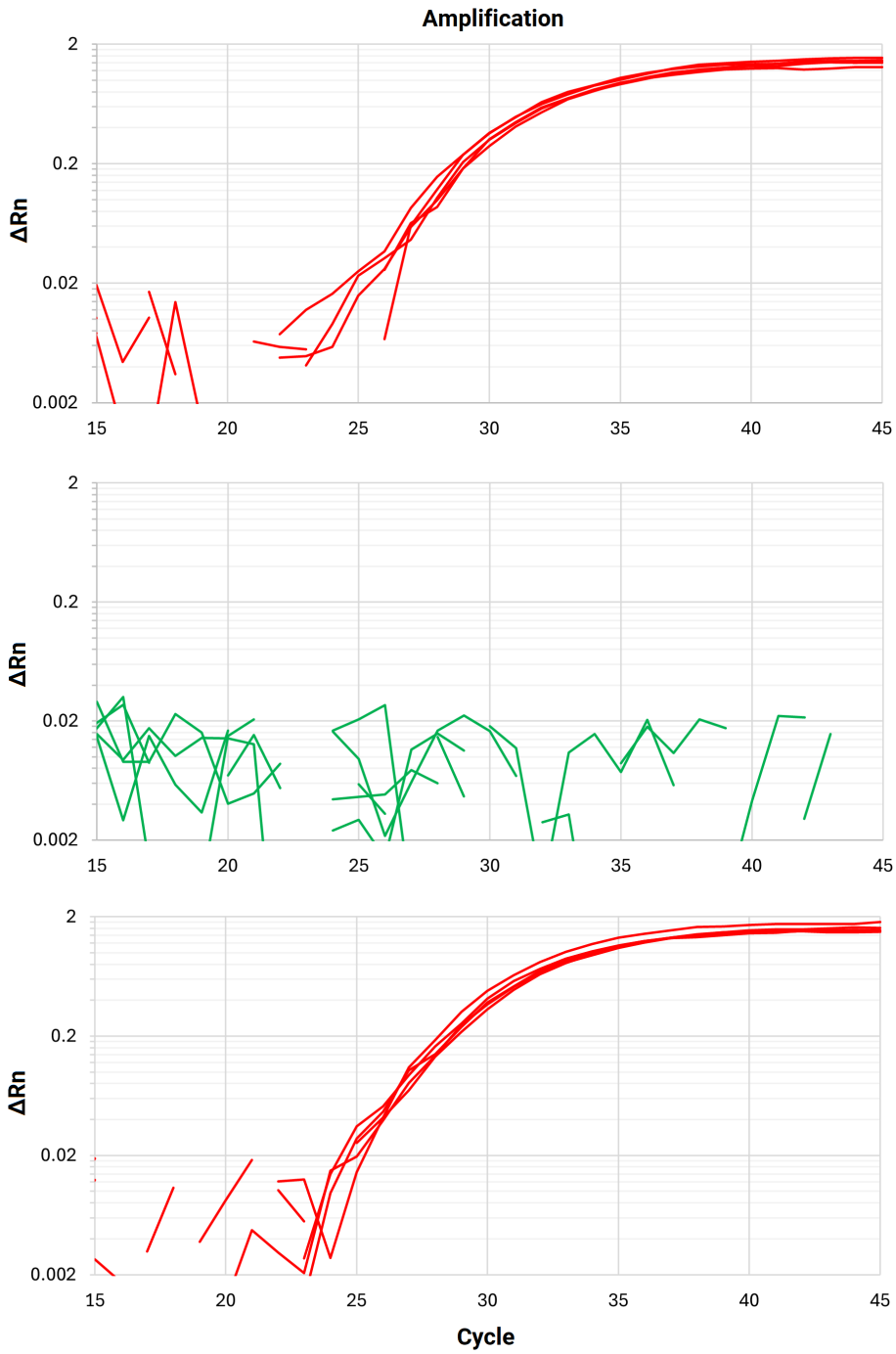
PCR amplification graph of change in fluorescence signal versus PCR cycle number. The 400 nL dispenses with the BioDot AD1520 are shown in red. Manually pipetted 5 µL reactions are shown in blue and no template controls are shown in green.

	Volume (µL)	Average Cq dispense	CV (%)	Number of replicates
BioDot AD1520 (Red)	0.4	26.563	1.75%	14
Manual (Blue)	5	26.373	0.872%	3

All reactions dispensed using the BioDot AD1520 system amplified with an average Cq of 26.56 (CV = 1.75%), indicating excellent consistency in low-volume dispensing. A manually pipetted control yielded an average Cq of 26.373 (CV = 0.872%), confirming comparable performance between automated and manual workflows. No-template control (NTC) wells showed no amplification, verifying that the system and reagents remained nuclease-free throughout the dispensing process.

Cross-Contamination Test

To verify cleaning effectiveness, alternating dispenses of DNA template and nuclease-free water were performed in a repeated pattern across multiple wells. No amplification was observed in water wells, and there was no drop in signal when reusing the same template, confirming cleaning reliability.



Series of qPCR reactions showing effectiveness of the bleach cleaning process. The top graph shows an initial set of qPCR reactions with DNA template added in. Cq = 27.0; N = 5 with CV of 1.16%.

After bleach cleaning, nuclease free water was added to PCR reactions instead of DNA template. The middle graph shows no amplification was observed. N = 5.

After bleach cleaning again, DNA template was added back into PCR reactions. The bottom graph shows amplification curves comparable to the first set. Cq = 26.9; N = 5 with CV of 0.92%.

Conclusion

BioDot's AD1520 enables nanoliter-scale qPCR with exceptional precision, minimal reagent use, and reliable cross-contamination prevention using fixed tips. With CVs under 2% and amplification performance matching manual controls, this method is ideal for labs seeking high-throughput, low-volume PCR without compromising data quality.

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