PCR

Comprehensive control of carryover contamination using heat-labile uracil-DNA glycosylase across PCR workflows

Enabling reliable amplification and product stability for downstream applications

Key findings

- Heat-labile uracil-DNA glycosylase (UDG) enables effective prevention of carryover contamination in PCR amplification methods
- The enzyme is inactivated at moderate temperatures, allowing controlled activity and compatibility with amplification processes
- Amplification products generated in the presence of heat-labile UDG remain stable and suitable for downstream applications
- Heat-labile UDG can be easily integrated into existing PCR workflows

Introduction

Carryover contamination from previously amplified products is a common challenge in PCR-based workflows and can lead to false-positive results, compromising assay reliability. A widely used strategy to address this issue involves incorporating dUTP during amplification, followed by treatment with uracil-DNA glycosylase (UDG) in subsequent PCR reactions to selectively degrade uracil-containing DNA.

UDG-based contamination control is routinely applied in a variety of nucleic acid amplification methods, including quantitative PCR (qPCR) and endpoint PCR. However, effective integration of UDG into different workflows requires careful control of enzyme activity to help ensure efficient degradation of contaminating DNA without affecting amplification performance or downstream applications.

Heat-labile UDG provides a versatile solution by enabling degradation of contaminating uracil-containing DNA prior to amplification while becoming inactivated during the thermal cycling process. This temperature-dependent inactivation prevents degradation of newly synthesized DNA and preserves amplification efficiency. Importantly, the use of heat-labile UDG allows amplification products to remain stable after the reaction, enabling their use in downstream applications such as re-amplification or further analysis.

In this application note, we evaluate the use of heat-labile UDG for carryover contamination control across one-step reverse transcription qPCR (RT-qPCR) and endpoint PCR amplification methods. We further assess enzyme inactivation characteristics and demonstrate the stability of amplification products generated in the presence of heat-labile UDG.

Materials and methods

Reagents

- Thermo Scientific™ Water, nuclease-free (Cat. No. R0581)
- Thermo Scientific™ Uracil-DNA Glycosylase, heat-labile (Cat. No. 78310100UN)
- Thermo Scientific™ dATP Solution (100 mM) (Cat. No. R0141)
- Thermo Scientific™ dCTP Solution (100 mM) (Cat. No. R0151)
- Thermo Scientific™ dGTP Solution (100 mM) (Cat. No. R0161)
- Thermo Scientific™ dUTP Solution (100 mM) (Cat. No. R0133)
- Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase (Cat. No. 14966001)
- Invitrogen™ Lyo-ready Platinum™ II *Taq* Hot-Start DNA Polymerase (Cat. No. EP225SMP2)
- Invitrogen™ Lyo-ready SuperScript™ III Flash Reverse Transcriptase (Cat. No. A40006521)
- Invitrogen™ RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019)
- Invitrogen™ Collibri™ DNA Library Cleanup Kit (Cat. No. A43190024)
- Invitrogen™ Universal Human Reference RNA for QuantiGene™ Assays (Cat. No. QS0639)
- Human genomic DNA
- Invitrogen™ TrackIt™ 100 bp DNA Ladder (Cat. No. 10488058)
- Invitrogen™ TrackIt™ Cyan/Orange Loading Buffer (Cat. No. 10482028)
- Primers and probe were custom designed at Thermo Fisher Scientific

Preparation of uracil-containing DNA amplicons

Uracil-containing DNA amplicons (spike) were generated for each amplification workflow to serve as model carryover contaminants to simulate contamination from previous reactions. In all cases, amplicons were produced using 100% dUTP in place of dTTP to enable selective degradation by UDG.

Reactions were assembled in 0.2 mL Applied Biosystems™ MicroAmp™ Optical 8-Tube Strip tubes and performed using the Applied Biosystems™ ProFlex™ PCR System. Reaction components and cycling conditions for each workflow are provided in Tables 1–4.

For endpoint PCR experiments, the spike was generated from human genomic DNA using PCR amplification under the conditions described in Tables 1 and 2.

For one-step RT-qPCR experiments, the spike was generated in a prior RT-PCR reaction using Universal Human Reference RNA (UHRR) as the template under the conditions described in Tables 3 and 4.

Following amplification, products were purified using magnetic beads (Collibri DNA Library Cleanup Kit) according to the "Remove EDTA from DNA samples" protocol in the user guide. Purified DNA concentrations were determined using a Thermo Scientific™ NanoDrop™ spectrophotometer and used as model carryover contaminants in subsequent experiments.

Assessment of heat-labile UDG inactivation

To assess the temperature-dependent inactivation of heat-labile UDG, PCR reaction mixes containing UDG (0.2 U per reaction) were prepared in the absence of template DNA and uracil-containing amplicon. PCR reaction mixes were prepared using standard PCR components as described for endpoint PCR.

Reaction mixes were pre-incubated at 50°C, 55°C, or 60°C for 5–20 minutes to induce UDG inactivation. Control reactions included UDG-containing samples without pre-incubation and reactions without UDG.

Following pre-incubation, 250 ng of a uracil-containing DNA amplicon was added to each reaction. Samples were then incubated at 37°C for 3 hours to allow residual UDG activity, followed by incubation at 95°C for 5 minutes to inactivate the enzyme and promote cleavage at abasic sites generated by UDG activity. Reaction products were analyzed by electrophoresis in TAE buffer on a 1% agarose gel stained with ethidium bromide.

Table 1. Endpoint PCR setup for dUTP-containing amplicon preparation.

Reagent	Final concentration
5X Platinum II PCR Buffer	1X
100 mM dATP	0.2 mM
100 mM dCTP	0.2 mM
100 mM dGTP	0.2 mM
100 mM dUTP	0.2 mM
Forward and reverse primers	0.2 µM each
Platinum II *Taq* Hot-Start DNA Polymerase	0.04 U/µL
Human genomic DNA template	20 ng/reaction
Water, nuclease free	Up to 20 µL

Table 2. Endpoint PCR cycling conditions.

Temperature	Time	Number of cycles	Step
94°C	2 min	1	Initial denaturation
94°C	15 sec	35	Denaturation
60°C	15 sec		Annealing
68°C	8 sec		Extension
4°C	Indefinitely		Hold

Table 3. One-step RT-PCR setup for dUTP-containing amplicon preparation.

Reagent	Final concentration
5X Lyo-ready Platinum II PCR Buffer	1X
50 mM $MgCl_2$	6.25 mM
100 mM dATP	0.8 mM
100 mM dCTP	0.8 mM
100 mM dGTP	0.8 mM
100 mM dUTP	0.8 mM
Forward and reverse primers	0.3 µM each
Lyo-ready Platinum II *Taq* Hot-Start DNA Polymerase (20 U/µL)	0.12 U/µL
Lyo-ready SuperScript III Flash Reverse Transcriptase (60 U/µL)	0.025 U/µL
RNaseOUT Recombinant Ribonuclease Inhibitor (40 U/µL)	0.4 U/µL
Universal Human Reference RNA template	1 ng/reaction
Water, nuclease free	Up to 20 µL

Table 4. One-step RT-PCR cycling conditions.

Temperature	Time	Number of cycles	Step
60°C	1 min	1	Reverse transcription
95°C	2 min	1	Initial denaturation
95°C	1 sec	40	Denaturation
60°C	15 sec		Annealing and extension

Evaluation of heat-labile UDG in endpoint PCR

To evaluate the performance of heat-labile UDG in endpoint PCR workflows, three experimental setups were used to assess carryover contamination removal, amplification performance, and stability of the products. Uracil-containing DNA amplicons were generated as described above and used to simulate carryover contamination. PCR reactions were performed using human genomic DNA (gDNA) as the template.

PCR reactions were assembled in 0.2 mL MicroAmp Optical 8-Tube Strip tubes according to the conditions described in Table 5. Reactions were performed using the ProFlex PCR System and amplified under the cycling conditions described in Table 2. Heat-labile UDG (0.2 U per reaction) was included where indicated. No-template control (NTC) reactions were performed to support the reliability of the results.

Reaction products were analyzed in TAE buffer by electrophoresis on a 1% agarose gel stained with ethidium bromide.

- **PCR sensitivity:** To evaluate the impact of UDG on amplification performance, PCR reactions containing gDNA template were performed in the presence or absence of UDG, with an additional incubation step for 5 min at 25°C prior to PCR cycling.
- **Carryover contamination removal:** PCR reactions were prepared in the absence of template DNA and supplemented with uracil-containing DNA spike. Reactions were performed in the presence or absence of UDG, with an additional incubation step for 5 min at 25°C prior to PCR cycling.
- **Stability of amplification products:** To assess the stability of amplification products generated in the presence of UDG, PCR reactions containing gDNA template and dUTP were performed with UDG under standard cycling conditions. Following amplification, reaction products were incubated at 25°C for 0–24 hours and subsequently subjected to incubation at 94°C for 5 minutes.

Table 5. Endpoint PCR setup for heat-labile UDG performance evaluation.

Reagent	Final concentration			
	Amplification of template DNA	Amplification of spike	Stability of amplification products	NTC
5X Platinum II PCR Buffer	1X	1X	1X	1X
100 mM dATP	0.2 mM	0.2 mM	0.2 mM	0.2 mM
100 mM dCTP	0.2 mM	0.2 mM	0.2 mM	0.2 mM
100 mM dGTP	0.2 mM	0.2 mM	0.2 mM	0.2 mM
100 mM dUTP	0.2 mM	0.2 mM	0.2 mM	0.2 mM
Forward and reverse primers	0.2 µM each	0.2 µM each	0.2 µM each	0.2 µM each
Lyo-ready Platinum II *Taq* Hot-Start DNA Polymerase (20 U/µL)	0.04 U/µL	0.04 U/µL	0.04 U/µL	0.04 U/µL
Uracil-DNA Glycosylase, heat-labile (1 U/µL)*	0.01 U/µL*	0.01 U/µL*	0.01 U/µL*	0.01 U/µL*
Human genomic DNA template	0.1–12.5 ng/reaction	–	0.5–10 ng/reaction	–
dUTP amplicon spike	–	0.01–1 pg/reaction	–	–
Nuclease-free water	Up to 20 µL	Up to 20 µL	Up to 20 µL	Up to 20 µL

* Endpoint PCR reactions were performed with and without uracil-DNA glycosylase.

Evaluation of heat-labile UDG in one-step RT-qPCR

To evaluate the performance of heat-labile UDG in one-step RT-qPCR workflows, experiments were designed to assess carryover contamination removal and the impact of UDG on amplification performance. Uracil-containing DNA amplicons were generated as described above and used to simulate carryover contamination. RT-qPCR reactions were performed using UHRR as the template.

RT-qPCR reactions were prepared in Applied Biosystems™ MicroAmp™ Fast Optical 96-Well Reaction Plates according to the composition described in Table 6. All compositions were tested without or with heat-labile UDG (0.2 U per reaction). A 25°C incubation step (5 minutes) was incorporated as the initial step of the RT-qPCR cycling protocol for all reactions to allow degradation of uracil-containing DNA. Reactions were amplified under the cycling conditions described in Table 7 using the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System. Amplification was monitored in real time using probe-based fluorescence detection. NTC reactions were included to monitor background amplification.

- **One-step RT-qPCR amplification performance:** RT-qPCR reactions were performed using only RNA template.
- **Carryover contamination removal:** RT-qPCR reactions were prepared in the absence of RNA template and supplemented with uracil-containing spike.
- **Impact of contamination on target detection:** RT-qPCR reactions containing both RNA template and spike were evaluated.

Table 6. One-step RT-qPCR setup for heat-labile UDG performance evaluation.

Reagent	Final concentration			
	Amplification of template RNA	Amplification of spike	Amplification of template RNA plus spike	NTC
5X Lyo-ready Platinum II PCR Buffer	1X	1X	1X	1X
50 mM $MgCl_2$	6.25 mM	6.25 mM	6.25 mM	6.25 mM
100 mM dATP	0.8 mM	0.8 mM	0.8 mM	0.8 mM
100 mM dCTP	0.8 mM	0.8 mM	0.8 mM	0.8 mM
100 mM dGTP	0.8 mM	0.8 mM	0.8 mM	0.8 mM
100 mM dUTP	0.8 mM	0.8 mM	0.8 mM	0.8 mM
Forward and reverse primers	0.3 µM each	0.3 µM each	0.3 µM each	0.3 µM each
Probe	0.2 µM	0.2 µM	0.2 µM	0.2 µM
Lyo-ready Platinum II *Taq* Hot-Start DNA Polymerase (20 U/µL)	0.12 U/µL	0.12 U/µL	0.12 U/µL	0.12 U/µL
Lyo-ready SuperScript III Flash Reverse Transcriptase (60 U/µL)	0.025 U/µL	0.025 U/µL	0.025 U/µL	0.025 U/µL
RNaseOUT Recombinant Ribonuclease Inhibitor (40 U/µL)	0.4 U/µL	0.4 U/µL	0.4 U/µL	0.4 U/µL
Uracil-DNA Glycosylase, heat-labile (1 U/µL)*	0.01 U/µL*	0.01 U/µL*	0.01 U/µL*	0.01 U/µL*
Universal Human Reference RNA template	0.01–1 ng/ reaction	–	0.01–1 ng/ reaction	–
dUTP amplicon spike	–	1 fg/reaction	1 fg/reaction	–
Nuclease-free water	Up to 20 µL	Up to 20 µL	Up to 20 µL	Up to 20 µL

* One-step RT-qPCR reactions were performed with and without uracil-DNA glycosylase.

Table 7. One-step RT-qPCR with UDG cycling conditions.

Temperature	Time	Number of cycles	Step
25°C	5 min	1	UDG activity
60°C	1 min	1	Reverse transcription
95°C	2 min	1	Initial denaturation
95°C	1 sec	40	Denaturation
60°C	15 sec		Annealing and extension

Results

Heat-labile UDG is inactivated at moderate temperatures

The temperature-dependent inactivation of heat-labile UDG was evaluated by pre-incubating the enzyme at 50–60°C for 5–20 minutes prior to addition of a uracil-containing DNA amplicon. Residual UDG activity was assessed based on its ability to degrade the spike (Figure 1). In the absence of pre-incubation, UDG efficiently degraded the uracil-containing DNA, resulting in no detectable product. In contrast, pre-incubation at 50–60°C resulted in loss of UDG activity, as indicated by the presence of intact DNA bands.

These results demonstrate that heat-labile UDG is effectively inactivated at moderate temperatures, enabling controlled removal of contaminating DNA prior to amplification while preventing degradation of newly synthesized products. This controlled activity is required for compatibility with PCR workflows and supports downstream applications requiring intact DNA.

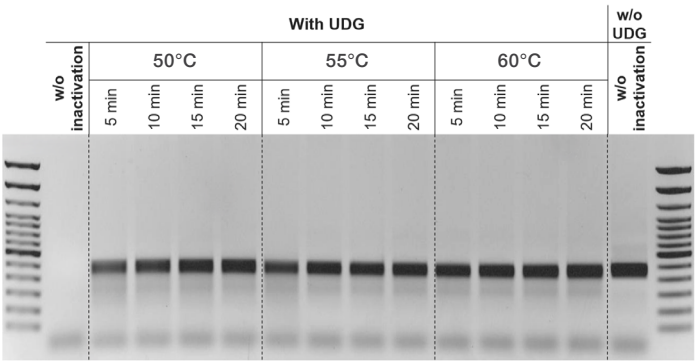

Figure 1. Inactivation of heat-labile UDG at moderate temperatures. Agarose gel analysis of uracil-containing DNA amplicon (0.5 kb, 250 ng) following incubation with heat-labile UDG (0.2 U/reaction) pretreated at different temperatures (50–60°C) and durations (5–20 min). Presence or absence of DNA target reflects residual UDG activity after thermal treatment. Samples without UDG and without pre-inactivation are shown as controls. DNA size standard: TrackIt 100 bp DNA Ladder.

Heat-labile UDG is compatible with endpoint PCR and minimizes carryover contamination

The compatibility of heat-labile UDG with endpoint PCR was evaluated using human gDNA across a range of input amounts. Comparable amplification products were observed in reactions performed with and without UDG, indicating that inclusion of UDG does not interfere with PCR amplification (Figure 2). No amplification was observed in NTC reactions.

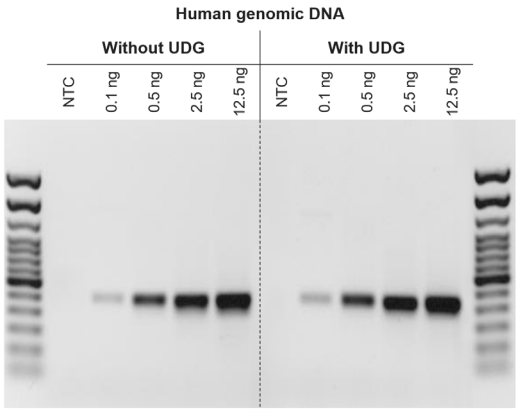

Figure 2. Effect of heat-labile UDG on endpoint PCR amplification of gDNA. Agarose gel analysis of PCR products generated from human gDNA input (0.1–12.5 ng) in the presence and absence of heat-labile UDG (0.2 U/reaction). Comparable amplification products (0.5 kb) are observed across input amounts. No amplification is observed in NTC reactions. DNA size standard: TrackIt 100 bp DNA Ladder.

To assess carryover contamination, uracil-containing DNA amplicons were introduced into PCR reactions in the absence of template DNA. In reactions without UDG, amplification products were detected for both spike inputs, demonstrating that contaminating amplicons can generate false-positive signals (Figure 3). In contrast, no amplification was observed in the presence of UDG, confirming efficient degradation of uracil-containing DNA and effective prevention of contamination-derived amplification.

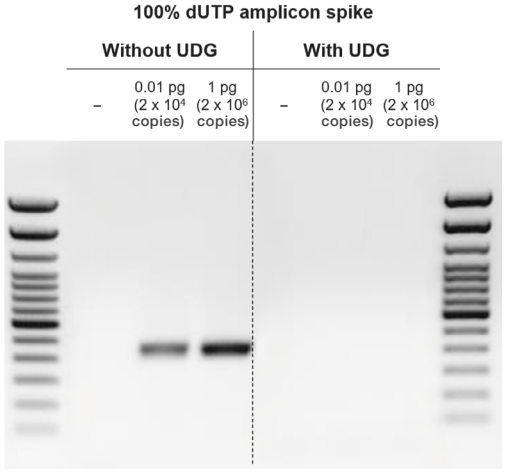

Figure 3. Elimination of carryover contamination in endpoint PCR using heat-labile UDG. Agarose gel analysis of PCR reactions containing uracil-containing DNA amplicon (0.01 pg and 1 pg, 2 x 10^4 and 2 x 10^6 copies, respectively) in the presence or absence of heat-labile UDG (0.2 U/reaction). Amplification products (0.5 kb) are observed in reactions without UDG, while no amplification is detected when UDG is included. DNA size standard: TrackIt 100 bp DNA Ladder.

These data indicate that heat-labile UDG can be readily integrated into endpoint PCR workflows to improve assay specificity without compromising amplification performance.

Amplification products generated with heat-labile UDG remain stable for downstream applications

To assess the stability of amplification products generated in the presence of UDG, PCR reactions containing human gDNA template and dUTP were performed with heat-labile UDG. Following PCR amplification, reaction products were incubated at 25°C for up to 24 hours and subsequently heated at 94°C for 5 minutes prior to analysis by agarose gel electrophoresis.

Amplification products remained intact across all incubation time points and input amounts, indicating that heat-labile UDG remains inactive after PCR and does not reactivate, thereby preventing degradation of amplified DNA (Figure 4).

This behavior highlights a key advantage of heat-labile UDG, as irreversible inactivation after amplification helps ensure that PCR products remain stable and suitable for downstream applications, including workflows requiring post-PCR processing or analysis.

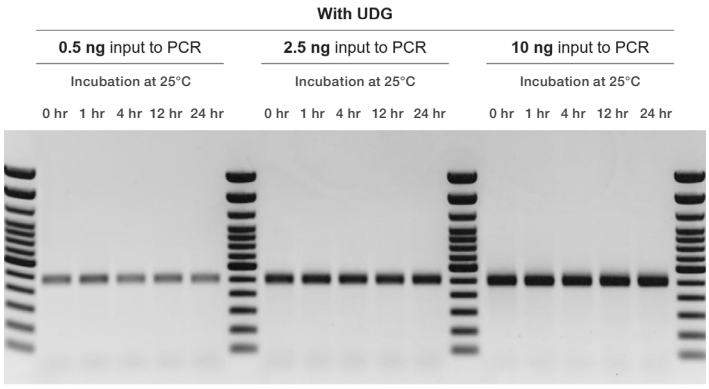

Figure 4. Stability of amplification products generated in the presence of heat-labile UDG. Agarose gel analysis of PCR products generated with heat-labile UDG (0.2 U/reaction) from various amounts of human gDNA (0.5–10 ng). Following PCR amplification, products were incubated at 25°C for up to 24 hours and subsequently heated at 94°C for 5 minutes prior to analysis. Amplification products (0.5 kb) remain intact across all conditions, indicating stability of DNA.

Heat-labile UDG enables reliable contamination control in one-step RT-qPCR

To evaluate the ability of heat-labile UDG to reduce carryover contamination, uracil-containing amplicons were introduced into RT-qPCR reactions in the absence of RNA template. In the absence of UDG, amplification was observed from the spike, demonstrating that contaminating amplicons can generate false-positive signals (Figure 5). Such false-positive amplification can compromise assay specificity and lead to misinterpretation of results, particularly in high-sensitivity workflows.

In contrast, no amplification was detected in reactions containing UDG, indicating efficient degradation of uracil-containing DNA. This demonstrates that heat-labile UDG effectively prevents amplification of carryover contaminants under one-step RT-qPCR conditions. No amplification was observed in NTC reactions.

These results confirm that heat-labile UDG effectively prevents contamination-derived amplification in RT-qPCR workflows, improving assay reliability.

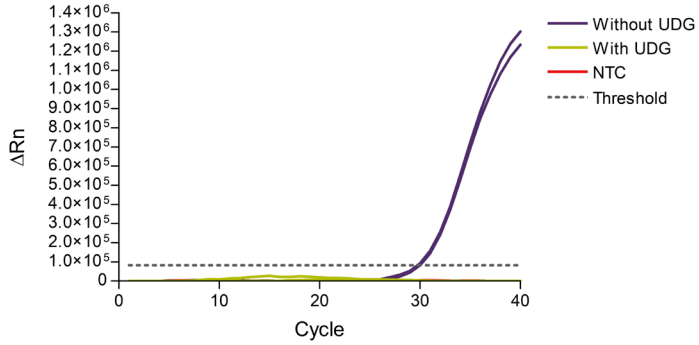

Figure 5. Elimination of carryover contamination in one-step RT-qPCR using heat-labile UDG. Real-time amplification curves from reactions containing 1 fg (1.3 x 10^4 copies) uracil-containing spike in the presence or absence of heat-labile UDG (0.2 U/reaction). Amplification is observed in reactions without UDG, while no amplification is detected when UDG is included. No amplification is observed in NTC reactions.

Heat-labile UDG preserves RT-qPCR performance

The impact of heat-labile UDG on RT-qPCR performance was evaluated using Universal Human Reference RNA (UHRR) across a range of input amounts. Comparable amplification curves were observed in reactions performed with and without UDG, indicating that inclusion of UDG does not interfere with reverse transcription or PCR amplification (Figure 6). C_t values were consistent between conditions, confirming that heat-labile UDG does not adversely affect assay sensitivity.

This demonstrates that heat-labile UDG is fully compatible with one-step RT-qPCR workflows, where preservation of reverse transcription efficiency is critical for accurate detection.

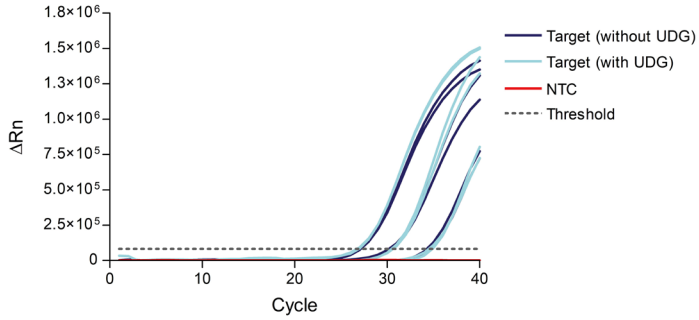

Figure 6. Effect of heat-labile UDG on RT-qPCR amplification of UHRR. Real-time amplification curves from one-step RT-qPCR reactions using UHRR at 0.01–1 ng per reaction, performed with and without heat-labile UDG (0.2 U/reaction). Similar amplification profiles are observed for both conditions. No amplification is observed in NTC reactions.

Heat-labile UDG restores accurate detection in contaminated samples

To assess the impact of carryover contamination on RT-qPCR results, reactions containing both RNA template and spike were analyzed. In the absence of UDG, amplification occurred at earlier cycles compared to RNA-only reactions, indicating contribution from contaminating amplicons (Figure 7). This shift in amplification profile can lead to overestimation of target quantity and reduced accuracy of quantification.

Inclusion of heat-labile UDG reduces the contamination-derived signal, restoring amplification profiles consistent with RNA-only reactions. This demonstrates that UDG not only removes contaminating DNA but also restores accurate target detection and quantification in the presence of carryover contamination.

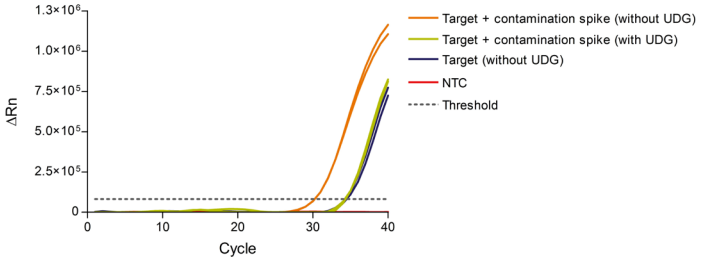

Figure 7. Restoration of target detection in contaminated RT-qPCR samples using heat-labile UDG. Real-time amplification curves from one-step RT-qPCR reactions containing UHRR (0.01 ng) with or without 1 fg (1.3 x 10^4 copies) uracil-containing spike, in the presence or absence of heat-labile UDG (0.2 U/reaction). Earlier amplification is observed in spike-containing reactions without UDG, while inclusion of UDG restores amplification profiles consistent with RNA-only reactions.

Conclusions

Heat-labile UDG offers a robust, streamlined solution for reliable carryover contamination control in PCR workflows. Through dUTP incorporation, it enables precise, selective degradation of contaminating amplicons, effectively reducing false positives and significantly enhancing assay specificity and data confidence. The enzyme is efficiently inactivated at moderate temperatures, enabling controlled degradation of uracil-containing DNA prior to amplification while preventing interference with newly synthesized products.

The results confirm that heat-labile UDG efficiently reduces contamination without affecting amplification efficiency, sensitivity, or overall assay performance. In addition, amplification products generated in the presence of UDG remained stable after PCR, showing that they can be used in downstream applications.

Together, these data demonstrate that heat-labile UDG can be easily integrated into PCR workflows to improve assay reliability, prevent false-positive results, and help preserve product integrity.

Learn more at **thermofisher.com/modifyingenzymes**