Isothermal amplification

Preventing carryover contamination in isothermal amplification using heat-labile uracil-DNA glycosylase

Key findings

- Heat-labile uracil-DNA glycosylase (UDG) enables effective prevention of carryover contamination in isothermal amplification workflows
- Contaminating amplicons are minimized even at relatively high input levels
- Enzyme is compatible with LAMP and RPA assays for sensitive detection of nucleic acid
- Simple format allows easy integration into existing isothermal amplification workflows

Introduction

Isothermal amplification methods such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) enable rapid and sensitive nucleic acid detection without the need for thermal cycling. These technologies are widely used in applications ranging from research to decentralized testing due to their speed, simplicity, and minimal instrumentation requirements.

However, the high sensitivity of isothermal amplification also increases susceptibility to carryover contamination from previously amplified products. Such contamination can result in false-positive signals, compromising assay reliability and limiting the robustness of these workflows, particularly in high-throughput or field-based settings.

A common strategy to prevent carryover contamination in standard PCR workflows involves incorporation of dUTP during amplification, followed by treatment with uracil-DNA glycosylase (UDG) to selectively degrade uracil-containing DNA. In isothermal amplification workflows, where reactions are performed at moderate, constant temperatures, effective contamination control requires an enzyme that is active prior to amplification but does not interfere with ongoing DNA synthesis. Heat-labile UDG offers a tailored solution by enabling degradation of contaminating uracil-containing DNA before amplification while becoming inactivated at the temperatures used for isothermal reactions.

This feature reduces degradation of newly synthesized DNA and allows compatibility with amplification enzymes, making heat-labile UDG particularly well suited for isothermal workflows.

In this application note, we evaluate the use of heat-labile UDG for carryover contamination control in LAMP and reverse transcription recombinase polymerase amplification (RT-RPA) workflows. The ability of UDG to reduce contaminating amplicons and its impact on assay performance were assessed across both amplification methods.

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™ Lyo-ready Bst DNA Polymerase (Cat. No. A56655)
- Invitrogen™ SYTO™ 9 Green Fluorescent Nucleic Acid Stain (Cat. No. S34854)
- Invitrogen™ Lyo-ready RPA Kit (Cat. No. A72127)
- Invitrogen™ SuperScript™ IV Reverse Transcriptase (Cat. No. 18090010)
- Invitrogen™ RNaseOUT™ Recombinant Ribonuclease Inhibitor (Cat. No. 10777019)
- Thermo Scientific™ RNase H, 5 U/µL (Cat. No. EN0201)
- Thermo Scientific™ DNA Loading Dye & SDS Solution (6X) (Cat. No. R1151)
- Invitrogen™ TrackIt™ 100 bp DNA Ladder (Cat. No. 10488058)
- Invitrogen™ Collibri™ DNA Library Cleanup Kit (Cat. No. A43190024)
- Invitrogen™ DNA*Zap*™ PCR DNA Degradation Solutions (Cat. No. AM9890)

Templates

- AMPLIRUN™ Adenovirus 41 DNA Control (GenBank sequence DQ315364.2) (Cat. No. MBC114-R, Vircell)
- Synthetic SARS-CoV-2 RNA Control 1 (GenBank sequence MT007544.1) (Cat. No. 102019, Twist Bioscience)

Primers

Primers for both LAMP (FIP/BIP, F3/B3, LoopF/LoopB) and RT-RPA (forward/reverse) were custom designed at Thermo Fisher Scientific.

Laminar flow hood cleaning

All reactions were assembled on ice in a laminar flow hood that was cleaned using 70% ethanol and DNA*Zap* PCR DNA Degradation Solutions.

LAMP reaction

To evaluate the effectiveness of heat-labile UDG in preventing carryover contamination, uracil-containing DNA amplicons were intentionally introduced into LAMP reactions to simulate contamination events. The ability of UDG to selectively degrade these contaminants while preserving amplification efficiency was assessed across multiple reaction conditions.

A uracil-containing DNA amplicon was generated in a prior LAMP reaction using 100% dUTP in place of dTTP and human adenovirus 41 (HAdV41) DNA template. This product served as a model carryover contaminant (spike) for subsequent experiments.

LAMP reactions for preparation of the dUTP-containing amplicon were assembled in 0.2 mL Applied Biosystems™ MicroAmp™ Optical 8-Tube Strip tubes according to the conditions described in Table 1. Reactions were incubated at 65°C for 30 minutes, followed by enzyme inactivation at 95°C for 2 minutes.

Table 1. LAMP reaction setup for dUTP-containing amplicon preparation.

Reagent	Final concentration
10X Bst Reaction Buffer	1X
5 M betaine	1 M
200 mM $MgCl_2$	6 mM
100 mM dATP	1.4 mM
100 mM dCTP	1.4 mM
100 mM dGTP	1.4 mM
100 mM dUTP	1.4 mM
FIP/BIP primers	1.6 µM each
F3/B3 primers	0.2 µM each
LoopF/LoopB primers	0.4 µM each
Lyo-ready Bst DNA Polymerase (6 U/µL)	0.24 U/µL
HAdV41 DNA template	100 copies/reaction
Water, nuclease free	Up to 25 µL

The resulting amplicons were purified using magnetic beads (Collibri DNA Library Cleanup Kit) following the "Remove EDTA from reaction" protocol. Purified DNA concentration was determined using a Thermo Scientific™ NanoDrop™ spectrophotometer.

To assess the ability of heat-labile UDG to reduce carryover contamination, LAMP reactions were prepared under the conditions described in Table 2. The following reaction types were evaluated, each performed in the presence or absence of heat-labile UDG (1 U/reaction):

- Template-only control to assess the impact of UDG on amplification sensitivity
- Spike-only reactions to simulate contamination in no-template control (NTC) samples
- Template + spike reactions to simulate contaminated target samples
- NTC

No additional pre-incubation step was required, as the enzyme was active under reaction setup conditions and effectively degraded uracil-containing DNA prior to amplification.

LAMP reactions were carried out under isothermal conditions as described in Table 3 using the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System. Amplification was monitored in real time via fluorescence of SYTO 9 stain, and melt curve analysis was performed following amplification to confirm product specificity.

Table 2. LAMP reaction setup for heat-labile UDG performance evaluation.

Reagent	Final concentration			
	Amplification of template DNA	Amplification of spike	Amplification of DNA template + spike	NTC
10X Bst Reaction Buffer	1 X	1 X	1 X	1 X
5 M betaine	1 M	1 M	1 M	1 M
200 mM $MgCl_2$	6 mM	6 mM	6 mM	6 mM
100 mM dATP	1.4 mM	1.4 mM	1.4 mM	1.4 mM
100 mM dCTP	1.4 mM	1.4 mM	1.4 mM	1.4 mM
100 mM dGTP	1.4 mM	1.4 mM	1.4 mM	1.4 mM
100 mM dUTP	1.4 mM	1.4 mM	1.4 mM	1.4 mM
FIP/BIP primers	1.6 µM (each)	1.6 µM (each)	1.6 µM (each)	1.6 µM (each)
F3/B3 primers	0.2 µM (each)	0.2 µM (each)	0.2 µM (each)	0.2 µM (each)
LoopF/LoopB primers	0.4 µM (each)	0.4 µM (each)	0.4 µM (each)	0.4 µM (each)
Lyo-ready Bst DNA Polymerase (6 U/µL)	0.24 U/µL	0.24 U/µL	0.24 U/µL	0.24 U/µL
Fresh 50 µM SYTO 9 stain solution	5 µM	5 µM	5 µM	5 µM
Uracil-DNA Glycosylase, heat-labile (1 U/µL)*	0.04 U/µL*	0.04 U/µL*	0.04 U/µL*	0.04 U/µL*
HAdV41 DNA template	100 copies/reaction	–	100 copies/reaction	–
dUTP amplicon spike	–	10 fg/reaction	10 fg/reaction	–
Nuclease-free water	Up to 25 µL	Up to 25 µL	Up to 25 µL	Up to 25 µL

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

Table 3. LAMP reaction conditions.

Temperature	Time	Number of cycles	Step
65°C	30 sec	60	Amplification
95°C	2 min	1	Inactivation
95°C	15 sec	–	Melt curve
60°C	15 sec		
95°C	15 sec		

RT-RPA reaction

To further evaluate the effectiveness of heat-labile UDG in isothermal amplification workflows, its ability to reduce carryover contamination was assessed in RT-RPA reactions. A uracil-containing DNA amplicon corresponding to the target sequence was generated in a prior RT-RPA reaction using 100% dUTP in place of dTTP and a SARS-CoV-2 RNA template, and used as a model carryover contaminant (spike).

RT-RPA reactions for preparation of the dUTP-containing amplicon were assembled in 0.2 mL MicroAmp Optical 8-Tube Strip tubes according to the conditions described in Table 4 and incubated at 42°C for 20 minutes.

Table 4. RT-RPA reaction setup for dUTP-containing amplicon preparation.

Reagent	Final concentration
2X RPA Reaction 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/reverse primers	0.3 µM each
SuperScript IV Reverse Transcriptase (200 U/µL)	2 U/µL
RNaseOUT Recombinant Ribonuclease Inhibitor	1.6 U/µL
RNase H (5 U/µL)	0.1 U/µL
Lyo-ready T4 UvsX Protein (1.2 mg/mL)*	0.09 mg/mL
Lyo-ready T4 UvsY Protein (1.2 mg/mL)*	0.03 mg/mL
Lyo-ready T4 Gene 32 Protein (16 mg/mL)*	0.4 mg/mL
Lyo-ready Bst DNA Polymerase (6 U/µL)*	0.15 U/µL
SARS-CoV-2 RNA template	40 copies/reaction
280 mM $MgCl_2$*	14 mM
Nuclease-free water	Up to 20 µL

* These components are included in the Lyo-ready RPA Kit. $MgCl_2$ was added last and separately to each sample ($MgCl_2$ initiates the reaction).

The amplified product was purified and quantified as described for the LAMP experiments. Later, the amplicon was introduced into RT-RPA reactions to evaluate the ability of heat-labile UDG to reduce contamination. RT-RPA reactions were prepared according to the conditions provided in Table 5.

The following reaction types were evaluated, each performed in the presence or absence of heat-labile UDG (1 U/reaction):

- Template-only reactions to assess the impact of UDG on amplification sensitivity
- Spike-only reactions to simulate contamination in NTC samples
- NTC

RT-RPA reactions were performed according to the conditions outlined in Table 6, including an incubation step for UDG activity, a UDG inactivation step, and an amplification step.

Amplification products were analyzed by DNA electrophoresis on a 2% TBE agarose gel containing ethidium bromide to assess the presence or absence of amplification products.

Table 5. RT-RPA reaction setup for heat-labile UDG performance evaluation.

Reagent	Final concentration		
	Amplification of template RNA	Amplification of spike	NTC
2X RPA Reaction Buffer	1 X	1 X	1 X
100 mM dATP	0.2 mM	0.2 mM	0.2 mM
100 mM dCTP	0.2 mM	0.2 mM	0.2 mM
100 mM dGTP	0.2 mM	0.2 mM	0.2 mM
100 mM dUTP	0.2 mM	0.2 mM	0.2 mM
Forward/reverse primers	0.3 µM each	0.3 µM each	0.3 µM each
SuperScript IV Reverse Transcriptase (200 U/µL)	2 U/µL	2 U/µL	2 U/µL
RNaseOUT Recombinant Ribonuclease Inhibitor	1.6 U/µL	1.6 U/µL	1.6 U/µL
RNase H (5 U/µL)	0.1 U/µL	0.1 U/µL	0.1 U/µL
Lyo-ready T4 UvsX Protein (1.2 mg/mL)	0.09 mg/mL	0.09 mg/mL	0.09 mg/mL
Lyo-ready T4 UvsY Protein (1.2 mg/mL)	0.03 mg/mL	0.03 mg/mL	0.03 mg/mL
Lyo-ready T4 Gene 32 Protein (16 mg/mL)	0.4 mg/mL	0.4 mg/mL	0.4 mg/mL
Lyo-ready Bst DNA Polymerase (6 U/µL)	0.15 U/µL	0.15 U/µL	0.15 U/µL
Uracil-DNA Glycosylase, heat-labile (1 U/µL)*	0.05 U/µL*	0.05 U/µL*	0.05 U/µL*
SARS-CoV-2 RNA template	5–100 copies/reaction	–	–
dUTP amplicon spike	–	1–10 fg/reaction	–
280 mM $MgCl_2$**	14 mM**	14 mM**	14 mM**
Nuclease-free water	Up to 20 µL	Up to 20 µL	Up to 20 µL

* RT-RPA reactions were performed with and without uracil-DNA glycosylase.

** $MgCl_2$ was added last and separately to each sample ($MgCl_2$ initiates the reaction).

Table 6. RT-RPA reaction conditions.

Temperature	Time	Step
25°C	10 min	UDG activity
50°C	1 min	UDG inactivation
42°C	20 min	RT-RPA amplification

Results

Heat-labile UDG reduces carryover contamination in LAMP

To assess the ability of heat-labile UDG to reduce carryover contamination, uracil-containing amplicons were introduced into LAMP reactions in the absence of target DNA. Without UDG, robust amplification was observed, demonstrating that high levels of contaminating amplicons can generate strong false-positive signals, highlighting the need for effective contamination control (Figure 1).

In contrast, inclusion of heat-labile UDG fully suppressed amplification from the spike, indicating efficient degradation of uracil-containing DNA prior to amplification. No amplification was observed in NTC reactions.

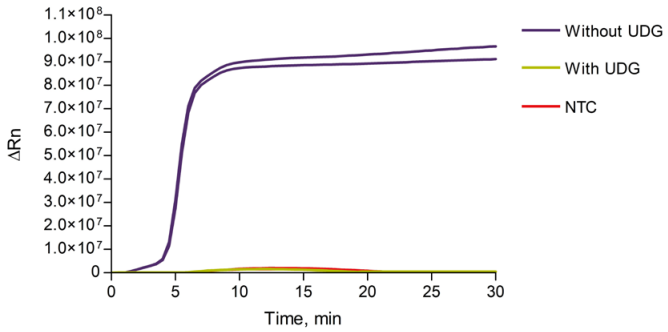

Figure 1. Amplification from uracil-containing spike in LAMP reactions with and without heat-labile UDG. Real-time amplification curves from LAMP reactions containing 10 fg uracil-containing amplicon spike in the presence or absence of heat-labile UDG (1 U/reaction). Amplification is observed without UDG but is suppressed when UDG is included.

Heat-labile UDG preserves LAMP assay performance

The impact of heat-labile UDG on LAMP assay performance was evaluated using HAdV41 DNA as the target in the presence and absence of contamination. Comparable amplification kinetics were observed for target-only reactions with and without UDG, indicating that UDG does not interfere with amplification efficiency (Figure 2).

In reactions containing both target DNA and spike, earlier amplification was observed in the absence of UDG, consistent with contribution from contaminating amplicons. Inclusion of UDG restored amplification profiles comparable to target-only reactions, demonstrating effective reduction of contamination without compromising assay performance.

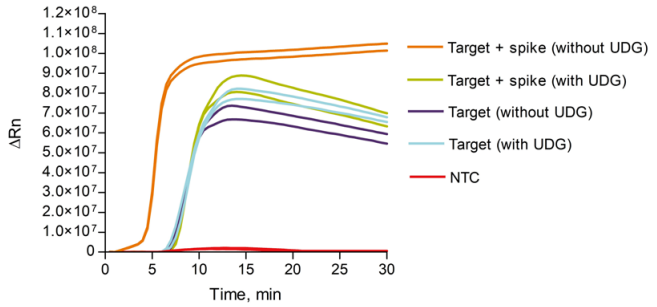

Figure 2. Effect of heat-labile UDG on LAMP amplification of HAdV41 DNA in the presence of spike contamination. Real-time amplification of HAdV41 DNA (100 copies per reaction) in the presence and absence of 10 fg spike, with or without heat-labile UDG (1 U/reaction). UDG restores amplification profiles by reducing contamination-derived signal.

Heat-labile UDG reduces carryover contamination in RT-RPA

The ability of heat-labile UDG to reduce carryover contamination was further evaluated in RT-RPA reactions using purified uracil-containing DNA amplicons generated from a prior RT-RPA reaction. In the absence of UDG, amplification products were observed for both 1 fg and 10 fg spike inputs, demonstrating that relatively high levels of contaminating DNA can generate robust amplification signals (Figure 3).

In contrast, no amplification products were detected in reactions containing UDG, indicating efficient degradation of contaminating DNA across the tested range. Reactions without added spike remained free of amplification.

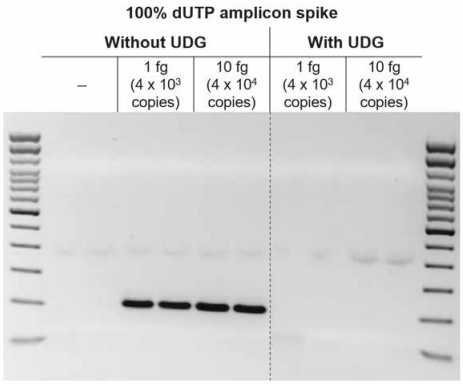

Figure 3. Agarose gel analysis of RT-RPA products from spike-containing reactions with and without heat-labile UDG. RT-RPA products (206 bp) generated from 1 fg and 10 fg (4.4×10^3 and 4.4×10^4 copies, respectively) uracil-containing amplicon spike in the presence or absence of heat-labile UDG (1 U/reaction). Amplification products are observed without UDG and are not present when UDG is included.

Heat-labile UDG maintains reliable RT-RPA detection

To assess the impact of heat-labile UDG on RT-RPA assay performance, amplification of SARS-CoV-2 RNA was evaluated across a range of input concentrations. Amplification products were observed in reactions performed both with and without UDG, confirming that target detection remained possible in the presence of heat-labile UDG (Figure 4).

Inclusion of UDG enables discrimination between true target amplification and signals arising from carryover contamination. In the absence of UDG, it is not possible to distinguish whether observed amplification originates from the target or from contaminating amplicons. While target band intensities were lower in reactions containing UDG, indicating a modest reduction in amplification efficiency, target detection remained clearly achievable. The reduced band intensity may reflect incomplete inactivation of UDG prior to RT-RPA amplification, as only limited inactivation conditions could be applied without compromising the reaction workflow. No amplification was observed in NTC samples.

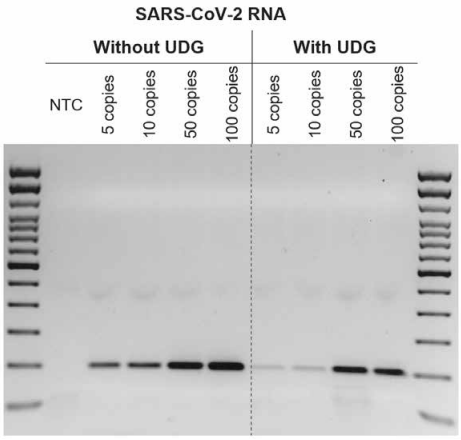

Figure 4. Effect of heat-labile UDG on RT-RPA amplification of SARS-CoV-2 RNA. Agarose gel analysis of RT-RPA products (206 bp) from SARS-CoV-2 RNA templates across different input concentrations (5–100 copies), with and without heat-labile UDG (1 U/reaction). Target amplification is observed in both conditions.

Conclusions

Heat-labile UDG offers an effective solution for preventing carryover contamination in isothermal amplification workflows, including both LAMP and RT-RPA. Incorporation of dUTP enables selective degradation of contaminating amplicons, reducing false-positive signals even at relatively high contamination levels. In LAMP reactions, heat-labile UDG helped reduce contaminating DNA without affecting amplification performance.

Heat-labile UDG also prevented carryover contamination in the RT-RPA workflow while maintaining reliable target detection These results demonstrate that heat-labile UDG can be readily integrated into isothermal amplification assays to improve reliability of the results and reduce the risk of false positives.

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