DNA/RNA Defend Pro™

PATHOGEN INACTIVATING AND STABILIZING LYSIS BUFFER FOR PRESERVATION OF DNA, RNA, AND ANTIGENS AND FOR EXTRACTION-FREE PCR

document reference: IB09 I117 DRDP R01 version 1.3
update: 17/01/2025

For research use only / For professional use only

INTENDED USE / GENERAL INFORMATION

DNA/RNA Defend Pro™ is a medium for pathogen inactivation, stabilization of RNA/DNA/antigens, and lysis of biological samples. The lysate can be used directly in PCR (or other enzymatic reactions) without time-consuming and expensive nucleic acid purification steps.

COMPOSITION

The product is an isotonic acidic salt solution supplemented with a nonionic surfactant and EDTA. The medium does not contain guanidine thiocyanate. A MSDS can be downloaded from the website www.inactivblue.com.

MATERIAL INCLUDED

DRDP_0100 1x 100 mL DNA/RNA Defend Pro™
DRDP_1000 1x 1000 mL DNA/RNA Defend Pro™

VARIANTS

The same medium, filled in a polypropylene tube, has been validated and CE-marked for clinical use with upper respiratory specimens (available as product code DRDP_0002). Reference is made to the product page of www.inactivblue.com.

MATERIAL NEEDED, BUT NOT PROVIDED

Sample collection device, such as a swab or saliva collection device (e.g. Blue Collect, product codeIB_CD01). Reference is made to the product page of www.inactivblue.com.

SPECIMEN TYPE

The uses of DNA/RNA Defend Pro™ are broad. Specimen types can include nasal swab, saliva, feces, urine, blood, tissue, pathogens, feed, food, cultured (single) cells, etc. It is strongly recommended to first test the sample type of interest with DNA/RNA Defend Pro™.

SUGGESTIONS FOR INITIAL SUITABILITY TESTS

As a starting point for such tests, it is recommended to use the following buffer vs. specimen ratios:

	DNA/RNA Defend Pro™	specimen quantity
cell pellet	1 mL	3 million cells
tissue, environmental samples	1 mL	100 mg
biofluids	1 mL	0.5 mL
swab	submerge the swab	1 swab
other	scale the recommended ratios proportionally	

NOTE: You may want to adjust the ratio of sample vs. buffer. If unsure, start with a larger volume of buffer relative to the sample (up to 9x) and work your way down to lower levels. Use at least 2x the volume of buffer relative to the sample volume.

STORAGE AND STABILITY OF DNA/RNA DEFEND PRO™ IN ITS ORIGINAL PACKAGING

- Store the product between 2 °C and 25 °C.
- Keep away from direct (sun) light.
- Shelf life: 24 months

INSTRUMENT/ SYSTEM COMPATIBILITY

DNA/RNA Defend Pro™ has been tested and found compatible with the following commercially available systems (other systems to be validated):

instrument(s)	nucleic acid extraction / (RT)-qPCR assays / master mixes
Alinity m (Abbott)	Multiplex Resp-4-Plex
geneXpert (Cepheid)	Xpert Xpress SARS-CoV-2/Flu/RSV assay
Panther Fusion (Hologic)	SARS-CoV-2/Influenza A/B/RSV assay
cobas Liat (Roche)	cobas SARS-CoV-2 & Influenza A/B cobas Influenza A/B & RSV
LightCycler 480 (Roche)	MagSi-NA Pathogens (magtivio, MDKT0021096) iTaq Universal One-step RT-qPCR kit (Bio-Rad)

instrument(s)	nucleic acid extraction / (RT)-qPCR assays / master mixes
	One Step PrimeScript III RT-PCR Kit (Takara Bio) PrimeTime One-Step 4x Broad-Range Master Mix (IDT) LightCycler Multiplex RNA Virus Master (Roche) TaqPath DuraPlex 1-Step RT-qPCR Master Mix (Applied Biosystems) TaqMan Fast Virus 1-Step Master Mix for qPCR (Applied Biosystems) eQo 1-Step ToughMix (QuantaBio)
Seegene STARlet (Seegene) CFX96 (Bio-Rad)	STARMag 96x4 Universal Cartridge Kit (Seegene) Allplex SARS-CoV-2/FluA/Flu B/ RSV assay (Seegene)

PERFORMANCE DATA

Below is a growing list of performance data generated in-house or by customers.

RNA / DNA STABILITY

- Bench tests have demonstrated that DNA/RNA Defend Pro™ successfully inhibits nucleases (using fluorescence release assay of quenched DNA or RNA oligonucleotide) (naturally present in saliva or swab or using spike-in RNase A).
- Numerous (RT-)qPCR tests have been performed in-house and by independent clinical labs to evaluate DNA and RNA stability in nasal swabs and saliva collected in DRDP™. The table below provides the outcome of tests performed on nasal swabs and saliva stored in DRDP™. DRDP™ stabilizes DNA / RNA up to 8 days after collection when stored between 2-25 °C (stability is defined as ≤ 2 Cq higher compared to day 0 in (RT)-qPCR).

Validation study demonstrated the following stability for the indicated targets:

	nasal swab		**saliva**	
assay / storage temperature	4 °C	25 °C	4 °C	25 °C
human DNA (GADPH, RSP18)	8 days	8 days	8 days	8 days
human RNA (CALR, TEMED2)	8 days	8 days	8 days	8 days
viral RNA (SARS-CoV-2 viral particles)	8 days	2 days	8 days	not determined

ANTIGEN STABILITY

- A series of experiments have been performed where DRDP™ was used instead of the standard buffer in rapid antigen tests. The low pH of the medium is not compatible with colloidal gold present in many lateral flow tests (precipitation can occur). Therefore, immediately before loading on a flow cell, the pH was neutralized. Study results show that nasal swab and saliva samples in DRDP™ can be reliably tested on at least the following commercially available tests (non-exhaustive list):
  - Flowflex, COVID19 Ag
  - Deepblue COVID19 Ag
  - Abbott COVID19 Ag
  - Fluorecare combi SARS-CoV-2, Flu A/B, RSV
  - Biosynex COVID19 Ag
  - Newgene COVID19
- Results demonstrate that DRDP™ preserves antigen epitopes in saliva and nasal swab material for at least 24 h.
- A clinical study on 23 swab samples demonstrated excellent agreement between nasal swab in DRDP™ and nasal swab in standard buffer in commercially available lateral flow tests and a very good concordance with results from RT-qPCR[1].
- In another retrospective paired study, 88 PCR-positive clinical swabs diluted in DRDP™ were retested on lateral flow tests (influenza, SARS-CoV-2, RSV). A good correlation was found between PCR and quick antigen test results. Samples with a negative antigen test had a significantly higher Cq or were PCR-negative. No false positives were found[2].

SUGGESTED METHOD FOR pH NEUTRALISATION:

- Aspirate 200 µL of the specimen using a single-use pipette (e.g. Thermo Scientific 941NL, #15377903) and dispense in a tube.
- Add 20 µL of a NaOH solution to the tube using a single-use pipette (e.g. Thermo Scientific 783NL, #1559524) and homogenize before loading on a flow cell.
- For swab and saliva samples, the concentration of NaOH to be added should be 0.70 and 0.47 M, respectively.

SUITABILITY FOR DIRECT PCR (i.e. EXTRACTION-FREE PCR)

- In a retrospective paired study, 88 PCR-positive clinical swabs diluted in DRDP™ were subject to extraction-free RT-qPCR. A good correlation was found: 72/88 (82%) scored also positive with direct PCR[2]. As expected, samples with a negative direct RT-qPCR result had a significantly higher Cq value when extracted and compared to samples with a positive direct PCR result (i.e. lower abundance of viral target), in line with a higher sensitivity obtained through extraction because of a 200-fold higher input compared to direct RT-qPCR. Further notes on reduced sensitivity in extraction-free testing are provided further.
- DRDP™ can be suitable for direct PCR in your specific test system upon validation. The maximum percentage of sample matrix will depend on the choice of master mix and target (PCR assay). On average, 15% of a sample can be added without signs of inhibition, but the range is 2.5 to 30%, as some master mixes are consistently more inhibitor-tolerant than others.
- Supplementing the PCR reaction with extra Mg^{++} is a simple and effective strategy to overcome some of the observed limitations of adding too much DRDP™ sample to the reaction.

SUGGESTED METHOD FOR INCREASING SAMPLE INPUT VOLUME

Increasing the Mg^{++} concentration in the PCR reaction by at least 0.4 mM per 10% of DRDP™ (added to the PCR reaction, v/v%) allows more sample input (up to 50%).

- e.g., add 1 µL Mg^{++} (30 mM) to a 25 µL PCR reaction to which 7.5 µL DRDP™ is added (30% DRDP™)
- e.g., add 0.5 µL Mg^{++} (80 mM) to a 25 µL PCR reaction to which 10 µL DRDP™ is added (40% DRDP™)

The optimal amount of Mg^{++} to be added depends on the sample matrix and the PCR reaction conditions (buffer and enzyme). Adding 0.4 to 1.0 mM per 10% DRDP™ is a good starting point. Lack of PCR inhibition with increasing amounts of sample can be evaluated as described in "SUGGESTED METHOD FOR EVALUATING DIRECT PCR SUITABILITY "

Study results indicated in the table below give an indication of the maximum percentage of sample matrix that can be directly applied in the following (RT-)qPCR reactions without inhibition: of note, no Mg^{++} was supplemented

sample type	**nasal swab**				**saliva (1:2 v:v)**			
target	endogenous RNA		spike-in		endogenous RNA		spike-in	
assay	18S	MALAT1	E-gene (RNA)	Lambda (DNA)	18S	MALAT1	E-gene (RNA)	Lambda (DNA)
PrimeTime One-Step 4x Broad-Range Master Mix (IDT)	10%	15%	15%	15%	20%	30%	5%	20%
LightCycler Multiplex RNA Virus Master (Roche)	5%	5%	10%	10%	20%	10%	15%	15%
TaqPath DuraPlex 1-Step RT-qPCR Master Mix (Applied Biosystems)	15%	15%	10%	<2.5%	30%	30%	30%	20%
TaqMan Fast Virus 1-Step Master Mix for qPCR (Applied Biosystems)	<5%	5%	5%	5%	25%	15%	30%	25%
One Step PrimeScript III RT-qPCR Mix (Takara)	15%	15%	10%	2.5%	20%	25%	25%	10%
eQo 1-Step ToughMix (QuantaBio)	15%	15%	15%	10%	15%	20%	20%	10%

- Direct PCR suitability should be confirmed with other PCR mixes and biomaterials.

SUGGESTED METHOD FOR EVALUATING DIRECT PCR SUITABILITY

It is recommended to set up a pilot study to determine if and to what level sample:buffer lysates are inhibitory. Inhibitor-tolerant (RT-)qPCR mixes should be used for maximal sensitivity (see higher). The following 2 experiments can be performed to verify absence of inhibition, or determine optimal amount of lysate in the PCR reaction:

- When lowering input of lysed sample from 10%, 5%, 2.5%, and 1.25% in the PCR reaction, a 2-fold, 4-fold, or 8-fold difference in signal should be observed, respectively (equivalent to a 1, 2, or 3 cycle difference, respectively). If this is not the case, the higher input amounts may inhibit the reaction.
- Add a purified DNA or RNA sample to the lysis buffer (1:2 v/v) and nuclease-free water (1:2 v/v) as a negative control, and then use 15%, 10%, 5%, 2.5%, and 1.25% of each of them separately as input in (RT-)PCR. Similar results for DNA/RNA diluted in lysis buffer or in water indicate lack of buffer inhibition

SUGGESTED METHOD TO MAXIMIZE LYSIS

Some cells/pathogens are harder to lyse than others, therefore, optimal lysis conditions depend on sample type. It is recommended to evaluate if additional steps are needed to maximize the lysis (e.g. mechanical mixing, vortexing, freeze/thaw cycles, heating, incubation with proteinase K).

Method:

1. Lyse sample by adding 1 mL of buffer to 0.5 mL of sample (or 1 mL lysis buffer per 100 mg sample, see higher).
   Note: ratio buffer-to-sample may need optimization for maximal efficiency
2. Optionally (especially for (pathogenic) microorganisms), add proteinase K to shorten the lysis duration and improve stability of the nucleic acids. The final concentration of proteinase K in the lysis reaction should be 50-400 µg/mL.
3. Incubate at 37 °C (up to 55° C) for at least 15 mins (up to 3 hours). Samples may be lysed overnight to be sure of complete nuclease digestion. Shaking, vortexing, or mixing helps the lysis procedure.
4. Inactivate proteinase K by heating to 95° C for 10 minutes after incubation.
   Note: This step can be omitted if no proteinase K is added
5. Cool lysate and store for later use or use directly in PCR (see step 6).
   Note: when sample is not completely lysed, a quick spin may bring debris to the bottom, to facility aspiration of supernatant as input in PCR
6. Avoid using more than 10% of pure lysis buffer as input in PCR, or more than 15% of lysate from a 1:2 sample:buffer mixture

   E.g. if 0.5 mL of sample is lysed with 1 mL of lysis buffer, then 3 µL of this lysate can be used in a 20 µL PCR reaction. If 100 mg of tissue is lysed in 1 mL lysis buffer, then maximally 1 µL can be used in a 20 µL PCR reaction.

   Note: Some PCR enzymes and buffer systems are more sensitive to possible inhibitors in the lysate; you may want to optimize the fraction of lysate added to your PCR reaction.

WARNING: While the use of a crude lysate into a nucleic test (without nucleic acid purification) comes with a reduction in cost, hands-on, and turnaround time, it may also come with a modest reduction in analytical sensitivity because the analyte of interest otherwise undergoes some level of up-concentration during a nucleic acid purification procedure. It is up to the user to determine if the advantage of using lysates outweighs the potential loss in analytical sensitivity. Such a loss can be determined by calculating the ratio between the sample input volume (into extraction) and the eluate volume (after extraction).

E.g. when using 0.5 mL of saliva into an RNA extraction procedure that elutes the RNA in 50 µl, a 10-fold nucleic acid concentration step is achieved. Hence, when using the same eluate volume or crude lysate volume into a PCR reaction, 10 times more target analyte is added to the PCR reaction when using purified RNA compared to crude lysate.

PATHOGEN INACTIVATION PERFORMANCE

- DNA/RNA Defend Pro™ effectively inactivates a broad range of pathogens. Test results are shown in the table below.

Table: Inactivation status of pathogens when 60 minutes exposed to DNA/RNA Defend Pro™

	complete inactivation			
RNA viruses	MERS-CoV	SARS-CoV-2	bRSV	H5N1
DNA viruses	mpox	vaccinia		
Gram+ bacteria	*S. pneumoniae*			
Gram- bacteria	*P. aeruginosa*	*E. coli*		

NOTE: The methods to determine pathogen inactivation performance are mentioned further.

SUGGESTED METHOD FOR VIRUS INACTIVATION TESTING

The TCID50 method can be used to determine viral inactivation performance[3,4]. TCID50 is defined as the dilution of a virus that infects 50% of a given cell culture. In the TCID50 assay, a highly concentrated viral stock is serially diluted, and each dilution placed on replicate cultures of susceptible, adherent cells in wells of a flat-bottomed plate. Infected cultures are incubated after which wells are scored positive or negative, based on the presence or absence of virally induced cytopathology (using microscopy).

Virus inactivation performance tests always include controls, including a cytotoxicity control.

Method

1. Mix virus-stock (preferably titer > 10^7 TCID50/mL) with DRDP™ in a 1:1 ratio
2. Further dilute virus:buffer mixture in culture medium: 10^{-1}, 10^{-2},10^{-3},10^{-4}, 10^{-5} , 10^{-6} , 10^{-7} ,10^{-8}, etc.
3. Incubate cells for 60 minutes in a 96-well, 5 wells per condition (200 µL/well)
4. Perform microscopy according to the TCID50 method

Control wells follow a similar protocol, with the following differences:

- For cytotoxicity control: cell culture medium + DRDP™ (1:1) and further dilutions (no viral stock)
- To determine residual titer: virus-stock + cell culture medium (1:1)
- Negative controls have cells in standard culture medium (culture medium + 2% fetal bovine serum + 1% antibiotic)

The table below indicates the level of cytotoxicity of DRDP™ buffer based on experimental data:

cells	dilution	DRDP™
MDBK	1:10	100% dead
	1:100	33-66% dead
	1:1000	0% dead
Vero E6 cells P33	1:10	100% dead
	1:100	0% dead
	1:1000	0% dead

After incubation, cell cultures are microscopically assessed for the presence or absence of cytopathic effect and TCID50 is calculated by the Reed and Muench method[5]. Depending on the type of cells, a dilution of 1:100 to 1:1000 is needed to eliminate the cytotoxicity of the DRDP itself. This implicates that if the TCID50/mL viral concentration at start would be TCID50/mL=$10^{7.5}$, and cytotoxic effects due to the medium are still observed at 2.5 log or 3.5 logs (i.e. TCID50/mL=$10^{2.5}$ or $10^{3.5}$ respectively), the maximum log reduction factor would be 5 logs or 4 logs respectively (7.5 minus 2.5 or 7.5 minus 3.5).

Although complete inactivation can be claimed, a greater log reduction cannot be quantified based on the starting concentration of the virus and given the fact that viral cytopathic effects could not be distinguished from cytotoxic effects by the buffer.

SUGGESTED METHOD FOR INACTIVATION TESTING OF BACTERIA / FUNGI

1. Culture strains of interest in appropriate growth media.
2. Verify if an ethanol treatment (100%) on bacterial/yeast suspension has a bactericidal effect. If so, ethanol 100% can be used as positive inactivation control.
3. Bring strains into suspensions at a concentration of log 7-9. We recommend testing a suspension (of bacteria/yeast), as well as a swab that is submerged in suspension (to mimic clinical sampling).
4. When testing a suspension, use at a ratio of 1:1. For a swab, submerge in the same volume of DNA/RNA Defend Pro™ as added to the suspension.
5. As positive controls, bring the bacterial/yeast suspension into saline (instead of test buffers).
6. As inactivation control, bring the bacterial/yeast suspension into ethanol 100%.

Table: Overview of conditions to test inactivation performance of DNA/RNA Defend Pro™

test item	negative control	positive control
suspension:DRDP (1:1)	suspension:saline (1:1)	suspension:ethanol 100% (1:1)
submerge swab in DRDP	submerge swab in saline	submerge swab in ethanol 100%

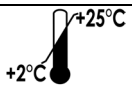
7. Incubate for 60 minutes or longer.
8. Plate a serial dilution series of the suspensions on suitable culture plates and incubate.
9. Count colonies and assess the bacterial/yeast concentration for each condition.

WARNINGS AND PRECAUTIONS

Harmful if swallowed, causes severe skin burns and eye damage. Wash hands and other exposed areas with mild soap and water before eating, drinking, or smoking and when leaving work. Provide good ventilation in process area to prevent formation of vapor.

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting viruses. Always wear protective clothing when handling specimens and reagent (gloves, lab vest, surgical mask, eye/face protection).

SYMBOL GLOSSARY

symbols as defined in ISO 15223			
	catalogue number		batch code
	use-by date		manufacturer
	keep away from (sun)light		temperature limit
	consult instructions for use		

BIBLIOGRAPHY

1. *Evaluation of a novel respiratory virus inactivating buffer for parallel RT-qPCR and quick antigen testing. medRxiv 2024. Deprez et al. (https://www.medrxiv.org/content/10.1101/2024.03.06.24303861v1)*
2. *Assessment of DNA/RNA Defend Pro: An Inactivating Sample Collection Buffer for Enhanced Stability, Extraction-Free PCR, and Rapid Antigen Testing of Nasopharyngeal Swab Samples. International Journal of Molecular Science 25(16), 9097; Claeys et al., 2024 (https://doi.org/10.3390/ijms25169097)*
3. *Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products, Transfusion (46) 1770-1777, Darnell & Taylor, 2006.*
4. *Inactivation of the coronavirus that induces severe acute respiratory syndrome, Journal of Virological Methods (121) 85–91, Darnell et al., 2004.*
5. *On the Calculation of TCID50 for Quantitation of Virus Infectivity. Virol. Sin. 36, 141–144. Lei, C., Yang, J., Hu, J. et al., 2021 (https://doi.org/10.1007/s12250-020-00230-5)*

TECHNICAL SUPPORT

URL: www.inactivblue.com
e-mail: info@inactivblue.com

MANUFACTURER INFORMATION

InActiv Blue bv
8730 Beernem (Belgium)
Tel: +32 50 791805
Fax: +32 50 791799