DNA/RNA Defend Pro™

VIRUS INACTIVATING AND STABILIZING LYSIS BUFFER FOR PRESERVATION OF DNA, RNA AND ANTIGENS

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For research use only

For professional use only

INTENDED USE / GENERAL INFORMATION

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

MATERIAL NEEDED, BUT NOT PROVIDED

Sample collection device

SPECIMEN TYPE

The uses of DNA/RNA Defend Pro™ are broad. Besides saliva and (naso/oro)pharyngeal swabs, other suitable specimen types can include feces, urine, blood, tissue, pathogens, feed, food, cultured (single) cells, etc. It is strongly recommended to first test the sample type of interest with DRDP.

SUGGESTIONS FOR INITIAL SUITABILITY TESTS

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

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).
- Results from qPCR (DNA) and RT-qPCR (RNA) indicate that endogenous RNA and DNA of human saliva and nasal swab material remain stable in DNA/RNA Defend Pro™ for 15 days at room temperature, and 21 days at 4 °C. Stability has been defined as the maximum time that ΔC_q remains ≤ 2 cycles compared to day 0.

Table : RNA and DNA target stability in DRDP buffer in function of sample type and storage temperature

specimen type	sample:DRDP ratio	spike	temperature (°C)	RNA stability (days)	DNA stability (days)
saliva	1:2	/	4	21	21
		/	21	15	15
		/	37	1	1
		SARS-CoV-2	4	21	n/a
nasal swab	submerged	/	4	21	21
		/	21	15	15

		/	37	7	7
		SARS-CoV-2	4	21	n/a

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ANTIGEN STABILITY

- A series of experiments have been performed where DNA/RNA Defend Pro™ was used instead of the standard buffer solution in quick antigen tests. Immediately before loading on a flow cell, pH was neutralized to avoid precipitation of the colloidal gold present in the flow cell. The results of these tests demonstrate that DRDP preserves antigen epitopes in saliva and swab material.
- This was further confirmed by ELISA tests where antigens were kept stable for at least 1 hour at 1:2 dilution (room temperature).

SUITABILITY FOR DIRECT PCR

- When spiked with purified DNA or RNA, DNA/RNA Defend Pro™ performs well in direct PCR experiments without signs of inhibition. Here we recommend max 10% of the DRDP buffer to be added to the (RT-)PCR reaction.
  NOTE: Direct PCR suitability needs to be further confirmed when using biomaterials (cells, stool, urine, saliva, swab, …) as input.

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 exposed to DNA/RNA Defend Pro™ for indicated time

			1 min	5 min	60 min
virus (titer of control > 10^7 TCID50/mL, unless indicated otherwise)	vaccinia		partial *virus log reduction = 5*	complete *virus log reduction ≥ 6*	complete *virus log reduction ≥ 6*
	bovine RSV (titer of control > 10^6 TCID50/mL)		complete *virus log reduction ≥ 5*	Complete *virus log reduction ≥ 5*	
	mpox (titer of control > 10^8 TCID50/mL)		partial *virus log reduction = 6*	partial *virus log reduction = 6*	complete *virus log reduction ≥ 7*
	influenza H5N1		partial *virus log reduction = 5*	complete *virus log reduction ≥ 6*	complete *virus log reduction ≥ 6*
	SARS-CoV-2		complete *virus log reduction ≥ 6*	complete *virus log reduction ≥ 6*	complete *virus log reduction ≥ 5*
bacterium	Gram-positive	*Staphylococcus aureus*		no inactivation	no inactivation
		Streptococcus pneumoniae		complete (no growth observed)	complete (no growth observed)
	Gram-negative	*Pseudomonas aeruginosa*		partial (log reduction 2-3)	complete (no growth observed)
		Escherichia coli		no inactivation	complete* (no growth observed)
	Mycobacterium smegmatis			no inactivation	no inactivation
fungus	*Candida albicans*			no inactivation	no inactivation

* Complete inactivation of bacteria on submerged swab, log reduction > 5 of bacteria brought in suspension

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 has not been determined yet.

SUGGESTED METHOD FOR EXTRACTION-FREE MOLECULAR ANALYSIS OF CRUDE LYSATES

The lysate from DNA/RNA Defend Pro™ can be used directly in PCR (or other enzymatic reactions) without time-consuming and expensive purification steps.

It has been extensively tested that the buffer does not inhibit (RT-)qPCR when present in maximally 10% of the enzymatic reaction. However, there may be additional sample-type dependent inhibition. Therefore, it is recommended to setup a pilot study in which you can determine if and to what level sample:buffer lysates are inhibitory.

The following 2 experiments can be performed to verify absence of inhibition, or determine optimal amount of lysate in the PCR reaction:

a) 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.
b) 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.

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 advantages 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.

SUGGESTED METHOD FOR PATHOGEN INACTIVATION TESTING

Viruses

The TCID50 method can be used to determine viral inactivation performance. 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 DNA/RNA Defend Pro™ 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 + DNA/RNA Defend Pro™ (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 shows the level of cytotoxicity of DNA/RNA Defend Pro™ buffer based on experimental data:

cells	dilution	DNA/RNA Defend Pro™
Vero E6	1:10	100% dead
	1:100	0-33% dead
	1:1000	0% dead
MDBK	1:10	100% dead
	1:100	33-66% dead
	1:1000	0% dead

In case the buffer itself is cytotoxic to the cells, the true effect of virus infection is masked. Therefore, dilutions of the buffer resulting in moderate (or worse) cytotoxicity in control wells (>66% cell dead), are excluded from the calculation for the log reduction factor (i.e. inability to conclude anything on viral infectivity).

Dilutions resulting in minimal cellular cytotoxicity in control wells, are included in the log reduction factor calculation when it is clear there is still 100% infection upon incubation with virus-spiked standard culture medium at the same dilution.

Example: DNA/RNA Defend Pro™ (DRDP) at a dilution down to 10^{-1} has severe cytotoxicity on Vero E6 cells (>66% dead). The cytotoxic effects are too pronounced to allow the observation of potential additional cytopathic effects imputable to the virus. Therefore, TCID50 below 10^{-1} cannot be claimed because nothing can be concluded about on viral infectivity. Hence, log (TCID50 of DRDP) = 1. If the residual titer is > 10^{7} TCID50/mL as determined in the controls (i.e. log (TCID50) is > 7), then, the virus log reduction is ≥ 6 for DRDP buffer.

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 to test both 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™ (DRDP) 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%

Note: to verify absence of contamination, we recommend to also culture DRDP separately.

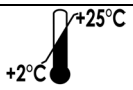
7. Incubate for 60 minutes or longer.
8. Plate a serial dilution series of the suspensions on suitable culture plates and incubate.
9. Count colonies and assess the bacterial/yeast concentration for each condition.

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 vapour.

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			
REF	catalogue number	LOT	batch code
	use-by date		manufacturer
	keep away from (sun)light		temperature limit
	consult instructions for use		

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TECHNICAL SUPPORT

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