DNA/RNA Defend™

PATHOGEN INACTIVATING AND STABILIZING LYSIS BUFFER FOR PRESERVATION OF DNA AND RNA

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For research use only

For professional use only

INTENDED USE / GENERAL INFORMATION

DNA/RNA Defend™ (DRD) is a medium for pathogen inactivation, stabilization of RNA and DNA, liquification of mucus-rich samples, and lysis of biological samples. It is the research-use-only and colorless version of CE-marked InActiv Blue© buffer.

COMPOSITION

The product contains guanidine thiocyanate and N-lauroylsarcosine. Complete product composition is described in the MSDS and can be downloaded from the website www.inactivblue.com

MATERIAL INCLUDED

DRD_0100 *1x 100 mL DNA/RNA Defend™*
DRD_1000 *1x 1000 mL DNA/RNA Defend™*

MATERIAL NEEDED, BUT NOT PROVIDED

Sample collection device

SPECIMEN TYPE

The uses of DNA/RNA Defend™ (DRD) are broad. Besides saliva and (naso/oro)pharyngeal swabs, other suitable specimen types 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 DRD.

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™	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™ 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) demonstrate that RNA and DNA of human saliva and nasal swab (both viral and endogenous) remain stable in DNA/RNA Defend™ for at least 30 days at 2-25 °C and 8 days at 37 °C. Stability has been defined as the maximum time that ΔC_q remains ≤ 2 cycles compared to day 0.
- DNA/RNA Defend™ is compatible with multiple freeze-thaw cycles without affecting DNA and RNA integrity.
- RNA remains intact when purified from human blood stabilized with DNA/RNA Defend™ at room temperature for at least 1 day
- RNA in human urine is stabilized for 9 days at 37 °C (RT-qPCR results upon RNA extraction)
- Tissues and biofluids stored in DNA/RNA Defend™ can be extracted with a wide variety of RNA and DNA extraction procedures (see InActiv Blue© buffer performance white paper on www.inactivblue.com).

PATHOGEN INACTIVATION PERFORMANCE

- DNA/RNA Defend™ 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™ for indicated time

			1 min	5 min	60 min
virus (titer of control > 10^7 TCID50/mL, unless indicated otherwise)	vaccinia			complete *virus log reduction ≥ 6*	complete *virus log reduction ≥ 6*
	norovirus				complete *virus log reduction ≥ 4-5*
	Carnivore protoparvovirus 1				partial *virus log reduction = 2.5*
	bovine RSV (titer of control > 10^6 TCID50/mL)			complete *virus log reduction ≥ 5*	complete *virus log reduction ≥ 5*
	mpox			complete *virus log reduction ≥ 7*	complete *virus log reduction ≥ 7*
	influenza H5N1			complete *virus log reduction ≥ 7*	complete *virus log reduction ≥ 7*
	SARS-CoV-2		complete *virus log reduction ≥ 6*	complete *virus log reduction ≥ 6*	complete *virus log reduction ≥ 6*
bacterium	Gram-positive	*Staphylococcus aureus*		complete* (no growth observed)	complete (no growth observed)
		Streptococcus pneumoniae		complete (no growth observed)	complete (no growth observed)
	Gram-negative	*Pseudomonas aeruginosa*		complete (no growth observed)	complete (no growth observed)
		Escherichia coli		complete (no growth observed)	complete (no growth observed)
	Mycobacterium smegmatis			complete (no growth observed)	complete (no growth observed)
fungus	*Candida albicans*			complete (no growth observed)	complete (no growth observed)

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

LIMITATIONS

- DNA/RNA Defend™ is not suitable for antigen preservation or extraction-free applications.
- DNA/RNA Defend™ receptacles cannot be decontaminated with bleach (hypochlorite) because the guanidine thiocyanate may form harmful cyanide gas after contact with hypochlorite. Therefore, DRD is not compatible with e.g. the Hologic Panther system.

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

- Store the product between 2 °C and 25 °C.
- Keep away from direct (sun) light.
- The medium remains stable for 24 months.

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™ 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™ (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 expected level of cytotoxicity of DNA/RNA Defend™ buffer based on experimental data:

cells	dilution	DNA/RNA Defend™
Vero E6	1:10	100% dead
	1:100	66-100% dead
	1:1000	0% dead
MDBK	1:10	100% dead
	1:100	33-66% dead
	1:1000	0% dead
CRFK	1:10	100% dead
	1:100	66-100% 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™ (DRD) at a dilution down to 10^{-2} 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^{-2} cannot be claimed because nothing can be concluded about on viral infectivity. Hence, log (TCID50 of DRD) = 2. 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 ≥ 5 for DRD 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™ (DRD) 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™

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

Note: to verify absence of contamination, we recommend to also culture DRD 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

DO NOT DECONTAMINATE TUBES OR LAB ENVIRONMENT WITH BLEACH! DNA/RNA Defend™ contains guanidine thiocyanate that in combination with hypochlorite may form harmful cyanide gas.

Harmful if swallowed, causes severe skin burns and eye damage, harmful to aquatic life with long lasting effects.

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	+25°C +2°C	temperature limit
	consult instructions for use		

BIBLIOGRAPHY

- *Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products, Transfusion (46) 1770-1777, Darnell & Taylor, 2006*
- *Inactivation of the coronavirus that induces severe acute respiratory syndrome, Journal of Virological Methods (121) 85–91, Darnell et al., 2004*

TECHNICAL SUPPORT

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