
INTERFERING SUBSTANCESNumber T-001 A

Introduction:

In the course of fermentation, downstream processing and formulation, a recombinant therapeutic product will encounter a wide range of biomolecules, buffers, solvents and formulation additives. The Threshold Total DNA Assay may be susceptible to inhibition from one or a number of these compounds. Thus, some samples to be tested for DNA, for process design or clearance, or release studies, will not be appropriate for direct assay in the Threshold System. Possible sources of interference must be recognized and then either be removed or neutralized (see Threshold Total DNA Assay Manual, Chapter 7). This note attempts to provide guidelines for acceptable levels of some commonly encountered compounds which may inhibit or interfere with accurate quantification of samples in the Threshold system.

Procedure:

Samples to be assayed on the Threshold system must meet the following requirements:

- 1) pH range: 6.8 - 8.0
- 2) Ionic Strength: 100 - 300 mM
- 3) DNA level: < 1 ng/ml
- 4) Sample volume: 500 μ l

If samples do not meet the above requirements, dilution, concentration, desalting, buffer exchange or other pretreatment methods may be required. A number of substances, commonly encountered in the processing or formulation of a therapeutic product or used to purify DNA, were tested in the Threshold system. Commonly encountered concentrations of each substance were tested in the Threshold system with a spike of 50 pg High Calibrator (Calf Thymus DNA) and without a spike (See Threshold Total DNA Assay Manual, Chapter 7). The buffer used in all cases, unless otherwise stated, is Zero Calibrator (50 mM PBS at pH 7.0 with 1 mM EDTA and 0.05 % Sodium azide). Acceptable spike recovery is generally considered to be 80 - 120 %. The results of this type of study reveal the maximum level at which each substance may be used in the Threshold system.

<u>POTENTIAL CONTAMINANT</u>	<u>CONCENTRATION¹</u>	<u>SPIKE RECOVERY/COMMENTS</u>
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Salts

Magnesium Chloride	10 mM**	82 %
	20 mM*	60 %
EDTA	10 mM*	100 %
MgCl ₂ /EDTA	20 mM/40 mM*	100 %
Ammonium Acetate	50 mM**	90 %;
(diluted from 2M stock)	100 mM	60 %; (Ionic Strength too high)
Ammonium Sulfate	50 mM**	126 %
(diluted from 2M stock)	100 mM	52 %; (Ionic Strength too high)

Protein denaturants

Proteinase K	100 µg/sample*	88 %
Dithiothreitol	50 mM*	100 %
Guanidine HCl	0.1 M	102 %
	0.5 M	43 %
Urea	10 mM**	80 %
	100 mM	45 %

Acids

Trifluoroacetic Acid	< 1 %	high background; pH too low
Acetic Acid	< 0.1 %	high background; pH too low

Polymers and Surfactants

Dextran	5 %*	100 %
Polyethylene glycol	5 %*	112 %
Dextran Sulfate	1 %**	62 %; causes high background
Triton X-100	2 %	100 %
SDS	0.1 %**	90 %
	0.2 %	50 - 80 %
SDS/Triton X-100	0.07 %/ 1.4 %	90 %

1 - The level listed is either the maximum level tested (*) or the maximum level acceptable (**)

<u>POTENTIAL CONTAMINANT</u>	<u>CONCENTRATION¹</u>	<u>SPIKE RECOVERY/COMMENTS</u>
<u>Organics</u>		
Methanol	20 %*	94 %
Ethanol	20 %*	90 %
Phenol	1 %**	92 %
	5 %*	cloudy and does not filter
Chloroform	Saturated*	94 %
Dimethylsulfoxide	5 %**	90 %
	10 %*	60 %
Acetonitrile	20 %**	100 %
	50 %*	0 %; Filtration rate increased
Mineral Oil	Saturated*	90 %
<u>Amino Acids</u>		
Arginine	10 mg/ml*	92 %
Glutamine	10 mg/ml*	97 %
Glycine	10 mg/ml*	81 %
Histidine	5 mg/ml **	90 - 100 %
Lysine	5 mg/ml**	90 - 100 %
<u>Carbohydrates</u>		
Glucose	10 mg/ml*	93 %
Mannitol	10 mg/ml*	86 %
Sucrose	10 mg/ml*	89 %
Glycogen	400 µg/ml*	100 %
<u>Other</u>		
Acetonitrile/TFA	20 %/0.1 %**	95 %
DNase I	12.5 µg/sample	100 %
MgCl ₂ /DNase I	5 mM/12.5 µg**	0% (desired); higher levels clog filter
tRNA	< 100 ng/sample**	> 80 %
	1 µg/sample*	54 %; possible DNA contamination
HSA or BSA	500 µg/sample**	100 %; direct assay, no pretreatment
Phenol Red	500 µg/sample*	100 %

The attached table lists the compounds studied, the maximum concentrations studied and the percent spike recovery. The compounds are classified according to common characteristics. Spike recovery values listed here should not be used directly to correct quantitation of unknowns. The user should assay control samples containing the potential interfering substance simultaneously with unknown samples to properly correct quantitation.

Conclusions:

The current list is by no means comprehensive. Any substance which may have one or more of the following characteristics may interfere with the Threshold system and should be thoroughly tested by performing spike recovery studies with a dilution series of commonly encountered concentrations.

- 1) an affinity for DNA or proteins
- 2) degrades proteins
- 3) inhibits or mimics Urease activity
- 4) mimics DNA
- 5) present in high concentration

Many substances are compatible with the Threshold Total DNA assay up to specified levels; however, it must be noted that, as of this date, these substances have not been tested for compatibility with either proteinase K or DNase I treatments. Therefore, it is important to validate substances which may interfere with pretreatment methods.

This application note should only be used as a guide in determining compatibility of potential interfering substances. Although a substance may be listed in this application note, the user should still personally evaluate the compatibility of all potential interfering substances in the Threshold assay.

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