

A phenol-free DNA extraction method

INTRODUCTION

A DNA extraction method was evaluated using a commercially available extraction kit for pretreatment of biopharmaceutical samples to be tested in the Threshold[®] Total DNA Assay. This extraction procedure incorporates a chaotrope, sodium iodide, an anionic detergent, sodium N-lauroyl sarcosinate, and isopropanol to co-precipitate nucleic acids with a polysaccharide carrier, glycogen. This precipitation allows the separation of DNA from sample components, proteins, lipids, and buffers that may interfere with the Total DNA Assay. For many proteins, this extraction technique can replace a conventional overnight digest with Proteinase K and SDS, and/or a phenol/chloroform extraction. The application note presents experiences of Threshold users and results of evaluation in our application laboratories.

MATERIALS

- 1 *Threshold*[®] *System* from Molecular Devices Corporation (catalog #0200-0500), 1311 Orleans Drive, Sunnyvale, CA 94089, tel: 408-747-1700 or 800-635-5577.
- 2 *Total DNA Assay Kit* from Molecular Devices Corporation (catalog #R9009).
- 3 *Total DNA Zero Calibrator Kit* from Molecular Devices Corporation (catalog #R8004)
- 4 *DNA Extractor Kit* from Wako Chemicals USA, Inc. (catalog #295-50201), 1600 Bellwood Rd., Richmond, VA 23237, tel: 800-992-9256, fax: 804-271-7791; or Wako Chemicals GmbH, Nissanstr. 2, W-4040 Neuss 1, Germany, tel: 49-2131-311154, fax: 49-2131-311100.

DNA Extractor Kit Chemical Components:

- Sodium Iodide Solution (26 mL)
 - Sodium N-Lauroyl Sarcosinate Solution (1.2 mL)
 - Washing Solution A (42 mL)
 - Washing Solution B (2x40 mL)
 - Glycogen Solution (0.1 mL)
- 5 *Isopropyl alcohol*, histological grade.
 - 6 *Microfuge* (X10,000g).

- 7 *Vortex mixer.*
- 8 *Sterile pipet tips.*
- 9 *Sterile, 2.0 mL conical tubes with o-ring* from Sarstedt, Inc. (catalog #72.693.005), P.O. Box 468, Newton, North Carolina 28658-0468, tel: 800-257-5101, fax: 704-465-4003.
- 10 *NUNC sterile 4.5 mL Cryotubes* from NUNC, Inc. (catalog #363452), 2000 North Aurora Road, Naperville, Illinois 60563, tel: 800-288-6862.
- 11 *Protein samples* were kindly provided by Molecular Devices Threshold customers.

METHODS

The extraction procedure is performed prior to heat denaturation of the DNA, therefore it is important to practice good aseptic technique to minimize DNA contamination. Sterile, wrapped tips, sterile tubes, and gloves should be used throughout this procedure. Where possible, sterile, individually wrapped Eppendorf Combitips[®] in combination with an Eppendorf Repeater Pipetter are recommended. One kit contains reagents for 50 sample extractions.

Assay procedure

- Step 1** Add 64 μL of Glycogen Solution to Sodium Iodide Solution¹.
- Step 2** Add 2 μL of Glycogen Solution₂ to one bottle of Washing Solution B and swirl gently to mix thoroughly.
- Step 3** Calculate the volume of Isopropanol needed for the day's extraction and aliquot into a sterile 50 mL tube.
- Step 4** Dispense 500 μL of sample into a sterile 2.0 mL Sarstedt microfuge tube with cap.
- Step 5** Add 20 μL of Sodium N-Lauroyl Sarcosinate Solution to the tube.
- Step 6** Add 500 μL of Sodium Iodide Solution (containing Glycogen Solution) to the mixture. Cap, vortex for 5-15 seconds, and incubate at 37-40°C for 15 minutes.
- Step 7** Add 900 μL of isopropanol (2-propanol) to the mixture using a 1 mL pipetter and a sterile tip. Little or no color change should occur. Vortex for 10-15 seconds and incubate at room temperature for 15 minutes.
- Step 8** Centrifuge (10,000xg) for 15 minutes in a microfuge to pellet the DNA. In some cases, a white pellet may be evident due to the Glycogen entrapping the precipitated DNA. Gently pour off the resulting supernatant, and blot tubes once on absorbent paper towel (dust-free lab wipes) to remove as much residual solution from the tubes as

¹ Crystals that may appear in the Sodium Iodide Solution during storage at 4°C can be redissolved by warming the solution to 50°C for a short period of time.

² The Washing Solution B is stable for only one week at 4°C after addition of the Glycogen Solution. If the Washing Solution B containing Glycogen Solution is not used within a week, the Glycogen Solution should be added again prior to using the Wash Solution B for an extraction.

possible. Use a clean portion of the towel for each tube. Do not tap the tubes on the paper towels, as this may dislodge the pellets.

- Step 9** Add 750 μL of Washing Solution A to the tube. Cap and mix for 5-15 seconds on a vortex mixer to resuspend the pellet in the tube.
- Step 10** Centrifuge for 5 minutes in a microfuge (10,000 \times g) and gently pour the resulting supernatant from the tube. Blotting is unnecessary.
- Step 11** Add 1500 μL of Washing Solution B containing Glycogen Solution to the tube. Cap and mix vigorously on the vortex mixer for 5-15 seconds.
- Step 12** Centrifuge for 5 minutes in a microfuge (10,000 \times g) and gently pour off the supernatant. Drain the tube well by blotting once on a clean paper towel.
- Step 13** Resuspend the remaining pellet (containing glycogen and DNA) in 0.5 mL Zero Calibrator. Mix on the vortex mixer to solubilize the pellet.
- Step 14** Heat the sample at 105°C for 15 minutes to denature the DNA. Chill on ice for 5 minutes and test in the Threshold Total DNA Assay.

ALTERNATE ASSAY PROCEDURES

Extraction of digested samples

If a sample does not filter properly or full spike recovery is not achieved after a direct extraction, an overnight digest with Proteinase K and SDS may be required. The protocol outlined in the *Threshold System Operator's Manual* specifies the addition of 25 μL Proteinase K at 2mg / mL and 25 μL of 2% SDS to a 0.5 mL sample, with and without DNA spike, followed by an overnight incubation at 55°C. If the digestion will be followed by a non-phenolic extraction, 25 μL of 20% SDS may be used to further aid digestion. An additional wash of the pellet is required if this extraction procedure follows a protein digest with 20% SDS:

- After digestion, extract the sample as outlined in the procedure through step #8. Resuspend the precipitated sample in 1 mL of Wash Solution B (Glycogen Solution added), and mix for 5-15 seconds on a vortex mixer.
- Centrifuge (10,000 \times g) the sample for 5 minutes, and gently pour off the supernatant. Proceed to step #9 in the standard procedure.

Extraction of sample volumes greater than 0.5 ml

Method A

Multiple extraction of 500 μL aliquots of a sample in the same tube. The first 500 μL aliquot can be treated as outlined in steps #1-8 in the procedure. A second 500 μL aliquot of sample is added to the same tube to resuspend the pellet, and steps #5-8 are repeated using Sodium Iodide Solution **without the Glycogen Solution added**. This procedure can be repeated to accommodate the required sample volume, however, background rates may increase. The final resulting pellet should be washed and resuspended in Zero Calibrator according to the procedure outlined in steps #9-14.

Method B

Increased sample volumes can be extracted with this method by increasing additions of the extractor kit components proportionally. Sterile, screw-cap NUNC vials can be used to accommodate the larger volumes, and an appropriate centrifuge rotor size must be selected. The final, precipitated pellet is suspended in 0.5 mL Zero Calibrator and transferred to a 2.0 mL sarstedt tube. (Note: to date only 1 mL sample volumes have been tested using this method.)

Both protocols described previously have been compared using two 0.5 mL sample aliquots and one 1 mL sample aliquot of monoclonal antibody at 5 mg/mL, and both produced acceptable spike recovery (Table 1).

	(+) Spike	(-) Spike	Net	% Spike Recovery
ZC (1 mL extraction)	60.5	11.3	49.2	–
Protein (1 mL extraction)	49.1	7.6	41.5	84
ZC (2x0.5 mL extraction)	56.2	4.2	52.0	–
Protein (2x0.5 mL extraction)	46.4	1.7	44.7	86

Table 1: Comparisons of methods for increased volume extraction

APPLICATIONS

A number of protein samples were extracted using this procedure and then tested in the Threshold Total DNA Assay. Samples were extracted and assayed in triplicate, with and without an addition of calf thymus DNA. The maximum amount of each sample extracted that allowed full spike recovery is listed in Table 2.

Sample	Maximum Level Tested	% Spike Recovery
Bovine Somatotropin	25 mg	105
Monoclonal antibody*	13.6 mg	99
Hemoglobin	20 mg	100
Gamma interferon	1 mg	99
Proteinase K	2 mg	91
Hirudin	100 mg	95
<i>rec</i> Staphylokinase	2 mg	93

Table 2: Samples tested with phenol-free DNA extraction method. *Sample was digested with 50 μ g Proteinase K and 0.1% SDS overnight before extraction

Several proteins (e.g. some monoclonal antibodies) can be extracted and assayed without a previous digestion with Proteinase K and SDS.

SUMMARY

This extraction method provides an alternative to a conventional phenol/chloroform extraction as a pretreatment of samples to be tested with the Threshold Total DNA Assay. For soluble proteins that do not ionically bind DNA, this extraction technique isolates the sample DNA by selective precipitation. This separates the sample DNA from interfering protein and buffer components, and allows the sample to then be resuspended in Zero Calibrator. This method is particularly effective when proteins exhibit a high pI and bind the sample DNA. The sample pH can be increased to or above the pI of the protein prior to extraction to reduce the ionic binding. A later adjustment of the pH to neutrality is not required since the extracted DNA is resuspended in Zero Calibrator. This extraction method also allows for testing of volumes larger than 0.5 mL, which can allow testing of a greater mass of sample per test.

REFERENCES

- 1 Ishizawa, M., Kobayashi, Y., Miyamura, T. and Matsuura, S. Simple procedure of DNA isolation from human serum. *Nucleic Acids Research*, **19**: 5792 (1991).
- 2 Maniatis, T., Fritsch, E.F., and Sambrook, J. Molecular Cloning - A Laboratory Manual, second edition. *Cold Spring Harbor Laboratory Press* (1989).

SALES OFFICES

United States & Canada
Molecular Devices
Tel. +1-800-635-5577
Fax +1-408-747-3601

Brazil
Molecular Devices Brazil
Tel. +55-11-3616-6607
Fax +55-11-3616-6607

China
Molecular Devices Shanghai
Tel. +86-21-6887-8820
Fax +86-21-6887-8890

Germany
Molecular Devices GmbH
Tel. +49-89/96-05-88-0
Fax +49-89/9-62-02-34-5

Japan
Molecular Devices Japan, Osaka
Tel. +81-6-6399-8211
Fax +81-6-6399-8212

Molecular Devices Japan, Tokyo
Tel. +81-3-5282-5261
Fax +81-3-5282-5262

South Korea
Molecular Devices Korea, LLC
Tel. +82-2-3471-9531
Fax +82-2-3471-9532

United Kingdom
Molecular Devices Ltd.
Tel. +44-118-944-8000
Fax +44-118-944-8001

www.moleculardevices.com

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