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Inhibitor removal from DNA extracts

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes how to remove inihibitory substances such as humic substances from DNA that has already been extracted. The protocol is formulated for an initial input of 100 µL of extracted DNA however it can be adjusted to any other volume as well with minor changes. A lot of the buffers can be found in the following patent https://patents.google.com/patent/US7459548B2/en

Guidelines

Follow general lab etiquette. Wear gloves to prevent contaminating the samples. Clean the workspace before starting with 80% EtOH.

Materials

Materials required:

Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable depending on the supply situation.

Chemicals:

Sodium phosphate dibasic

Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S0876-100G

Guanidinium thiocyanate Guanidinium thiocyanate **Fisher Scientific Catalog #**10503345

Sodium phosphate monobasic Sodium phosphate monobasic

Sodium phosphate monobasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S0751-100G

SDS ultrapure Sodium dodecyl sulfate **Diagonal Catalog #**A1112.0500

Sodium chloride Sodium chloride **Fisher Scientific Catalog #**10616082

Tris ultrapure 99.9% Tris ultrapure 99.9% **Diagonal Catalog #**A1086.1000

Hydrochloric acid fuming 37%

Hydrochloric acid fuming 37% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**1003171011

Ammonium acetate Ammonium acetate **Carl Roth Catalog #**7869.2

Aluminium ammonium sulfate dodecahydrate

Aluminium ammonium sulfate dodecahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A2140-500G

Guanidine hydrochloride Guanidine hydrochloride **Fisher Scientific Catalog #**10543325

Acetic acid Acetic acid **Carl Roth Catalog #**7332.1

Ethanol absolute Ethanol absolute 99.8% p.a. **Carl Roth Catalog #**9065.1

Labware:

2 mL centrifuge tubes Reaction tube, 2 mL, PP **Sarstedt Catalog #**72.691

1.5 mL centrifuge tubes Reaction tube, 1.5 ml, PP **Sarstedt Catalog #**72.690.001

The EconoSpin® All-In-One DNA Only Mini Spin Column

The EconoSpin® All-In-One DNA Only Mini Spin Column **Epoch Life Science Catalog #**1920-250

Stock solutions:

1 L SDS stock solution [M] 10 Mass / % volume

- Add 100 g SDS ultrapure to a beaker

- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L sodium chloride stock solution [M] 5 Molarity (M)

- Add 292.2 g sodium chloride to a beaker
- Adjust volume to 1 L with ddH2O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution [M] 1 Molarity (M) pH 8

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH2O
- Adjust pH to pH 8 with HCl
- Adjust volume to 1 L with ddH2O
- Sterilize by filtering and store at Room temperature

500 mL sodium acetate stock solution [M] 3 Molarity (M) pH 5

- Add 123 g sodium acetate to a beaker
- Adjust volume to 400 mL with ddH2O
- Adjust ph to pH 5 with acetic acid
- Adjust volume to 500 mL with ddH2O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution [M] 1 Molarity (M) pH 7.5

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH2O
- Adjust pH to pH 7.5 with HCl
- Adjust volume to 1 L with with ddH2O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution [M] 1 Molarity (M) pH 8.5

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH2O

- Adjust pH to pH 8.5 with HCl
- Adjust volume to 1 L with with ddH2O
- Sterilize by filtering and store at Room temperature

1 L wash buffer stock solution ([M] 50 millimolar (mM) Tris) pH 7.5

- Add 50 mL Tris stock solution pH 7.5 to a beaker
- Adjust volume to 1 L with with ddH2O
- Sterilize by filtering and store at Room temperature

Working solutions:

500 mL bead-beating solution ([M] 180 millimolar (mM) sodium phosphate , [M] 120 millimolar (mM) guianidinium thiocyanate) pH 8

- Add 12.8 g sodium phosphate dibasic to a beaker
- Add 7.1 g guanidinium thiocyanate
- Adjust volume to 490 mL with ddH_2O
- Adjust pH to pH 8 by adding sodium phosphate monobasic
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

500 mL lysis solution ([M] 150 millimolar (mM) sodium chloride , [M] 4 Mass / % volume SDS , [M] 500 millimolar (mM) Tris) pH 8

- Add 200 mL of [M] 10 Mass / % volume SDS stock solution to a beaker
- Add 15 mL of [M] 5 Molarity (M) sodium chloride stock solution
- Add 250 mL of [M] 1 Molarity (M) Tris stock solution pH 8
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

500 mL ammonium acetate buffer ([M] 130 millimolar (mM) ammonium acetate)

- Add 5 g ammonium acetate to a beaker
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

500 mL inhibitor removal solution ([M] 120 millimolar (mM) aluminum ammonium sulfate dodecahydrate)

- Add 27.2 g aluminium ammonium sulfate dodecahydrate to a beaker
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

500 mL DNA binding buffer ([M] 2.5 Molarity (M) Guanidine hydrochloride , [M] 80 % (v/v) ethanol , [M] 0.05 % (v/v) Tween 20 , [M] 120 millimolar (mM) sodium acetate) pH 5

- Add 119.4 g guanidine hydrochloride to a beaker
- Fill up to 400 mL ethanol
- Add 20 mL [M] 3 Molarity (M) sodium acetate stock solution pH 5
- Adjust volume to 500 mL with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L wash buffer ([M] 10 millimolar (mM) Tris , [M] 80 % (v/v) Ethanol) pH 7.5

- Add 200 mL was buffer stock solution
- Adjust volume to 1 L with Ethanol absolute
- Sterilize by filtering and store at Room temperature

1 L elution buffer ([M] 10 millimolar (mM) Tris) pH 8.5

- Add 10 mL Tris stock solution pH 8.5 to a beaker
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

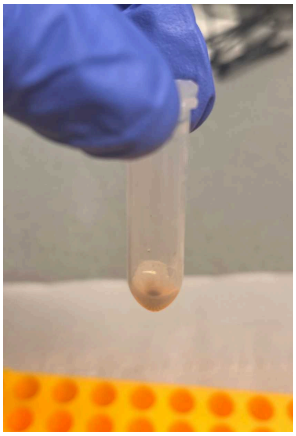
Before start

Make sure all buffers are prepared before starting.

Inhibitor removal from DNA extracts

31m

1 Prepare 100 µL of sample in 2 mL tubes.



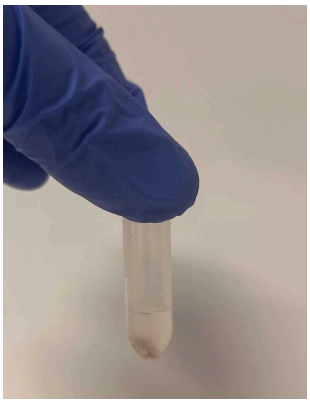
Sample contaminated with inhibitory substance (e.g. humic acids)

Note

This protocol is designed for a sample volume of 100 µL . Differing volumes can be used, however, all the following volums have to be adjusted accordingly. For volumes smaller than 100 µL we recommend to simply fill up the sample to 100 µL with water and then proceed with the protocol. If a sample volume of 200 µL is to be processed, all volumes have to be doubled.

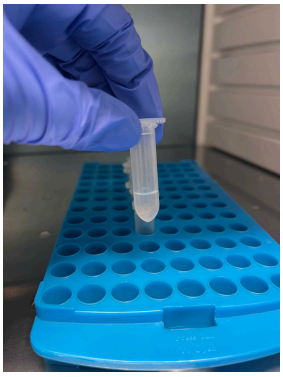
2 Add 345 µL bead-beating solution and 60 µL lysis solution . 10m
Vortex shortly.

3 10000 x g, 20°C , 00:03:00 . Transfer all of the supernatant to a new tube. 3m



Try to avoid the the pellet if any is formed.

4 Add 125 µL ammonium acetat buffer , vortex shortly and incubate at 4 °C for 00:05:00 . 5m



5 10000 x g, 20°C, 00:01:00 . Transfer the supernatant to a new tube. 1m

6 Add 100 µL of inhibitor removal buffer . A precipitate may form. Vortex shortly, incubate at 4 °C for 00:05:00 5m

7 10000 x g, 20°C, 00:01:00 . Transfer 600 µL of the supernatant to a new tube. 1m

8 Add 1200 µL DNA binding buffer . Vortex to mix.

9 Load 650 µL of the mixture to a mini spin column (e.g. Epoch Life Science).

10 10000 x g, 20°C, 00:00:30 . Discard the flow-through. Repeat two times to bind the complete sample volume. 30s

11 Add 500 µL wash buffer . 10000 x g, 20°C, 00:00:30 to wash the column. Discard the flow-through. 30s

12 10000 x g, 20°C, 00:01:00 to dry the column. Transfer the spin column to a clean 1.5mL microcentrifuge tube. 1m

13 Add 50 µL elution buffer . Incubate for 00:03:00 at Room temperature . 3m

14 10000 x g, 20°C, 00:01:00 to eluate the DNA. DNA eluate should be completely colorless and ready to go for downstrom analysis. 1m