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Version 1

# 🌐 Guanidine-based DNA extraction with silica-coated beads or silica spin columns V.1

📁 In 2 collections

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**We use this protocol and it's working**

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## Abstract

This protocol describes how to extract DNA from samples lysed as described in

### Protocol



NAME

**Sample preparation and lysis of homogenized malaise trap samples**

CREATED BY

**Dominik Buchner**

Preview

using guanidine hydrochloride and ethanol-based buffer combined with silica-coated magnetic beads or silica spin columns. The spin column protocol can be used either with centrifugation or, alternatively, a vacuum manifold. Compared to approaches with magnetic beads, with silica spin column protocols higher yields are possible since the amount of lysate used can be increased. The bead-based protocol is an automation-friendly alternative.

## Guidelines


Follow general lab etiquette. Wear gloves to prevent contamination of samples. Clean the workspace before starting and after finishing 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:

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

Bis-Tris  Bis-Tris **Carl Roth Catalog #9140.1**

Ethanol absolute  Ethanol absolute 99.8% **Fisher Scientific Catalog #11994041**


Phenol red indicator solution

 Phenol red indicator solution **VWR International (Avantor) Catalog #HACH21132**

Hydrochloric acid fuming 37%


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

SeraSil-Mag 400 silica-coated beads

 SeraSil-Mag 400 silica coated superparamagnetic beads **Merck MilliporeSigma (Sigma-Aldrich) Catalog #GE29357371**


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

EDTA disodium salt  EDTA disodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E5134-50G**


Sodium hydroxide  Sodium hydroxide - pellets **Fisher Scientific Catalog #S/4920/60**


### Labware:

50 mL Falcon tube

 Easy Reader Conical Polypropylene Centrifuge Tube **Fisher Scientific Catalog #11512303**

125 mL Nalgene Wide-Mouth Bottle


 Thermo Scientific Nalgene Wide-Mouth LDPE Bottle with Closure **Fisher Scientific Catalog #10044180**


Large magnet  Neodyme magnet **Magnethandel Catalog #3935**

1.2 mL square-well plate  1.2 mL square-well storage plate **Thermo Fisher Scientific Catalog #AB1127**

96-well plate magnet  MM-Separator M96 **Carl Roth Catalog #2141.1**

Hard-Shell PCR Plate  Hard-Shell 96-well PCR plate **Bio-Rad Laboratories Catalog #HSP9601**





EconoSpin mini spin column  EconoSpin mini spin column with lid **Epoch Life Science Catalog #1920-050**

1.5 mL Microcentrifuge tubes  1.5 mL microcentrifuge tube **Sarstedt Catalog #72,690,001**






EconoSpin 96-well filter plate  EconoSpin 96-well filter plate **Epoch Life Science Catalog #2020-001**

### Stock solutions:






 50 mL Bis-Tris stock solution [M] 1 Molarity (M)

- Add  10.5 g Bis-Tris to a  50 mL Falcon tube
- Adjust volume to  50 mL with ddH<sub>2</sub>O
- Vortex to completely dissolve the Bis-Tris
- Store at  4 °C






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

- Add  121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to  800 mL with ddH<sub>2</sub>O
- Adjust pH to  8.5 with HCl
- Adjust volume to  1 L with ddH<sub>2</sub>O
- Sterilize by filtering and store at  Room temperature




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

- Add  121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to  800 mL with ddH<sub>2</sub>O
- Adjust pH to  8 with HCl
- Adjust volume to  1 L with ddH<sub>2</sub>O
- Sterilize by filtering and store at  Room temperature

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





- Add  121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to  800 mL with ddH<sub>2</sub>O
- Adjust pH to  7.5 with HCl
- Adjust volume to  1 L with ddH<sub>2</sub>O
- Sterilize by filtering and store at  Room temperature

 1 L EDTA stock solution [M] 0.5 Molarity (M)  8






- Add  186.12 g EDTA disodium salt to a beaker
- Adjust volume to  1 L with ddH<sub>2</sub>O
- Adjust pH to  8 with sodium hydroxide









- Sterilize by filtering and store at  Room temperature



 1 L wash buffer stock solution (  50 millimolar (mM) Tris )  7.5







- Add  50 mL Tris stock solution  7.5 to a beaker
- Adjust volume to  1 L with ddH<sub>2</sub>O
- Sterilize by filtering and store at  Room temperature


### Working solutions:





 1 L GuHCl binding buffer (  3 Molarity (M) Guanidine hydrochloride ,  10 millimolar (mM) Bis-Tris  90 % (v/v) Ethanol )  6





- Add  286.6 g Guanidine hydrochloride in a beaker
- Adjust volume to  900 mL with Ethanol absolute
- Add  10 mL Bis-Tris stock solution
- Adjust volume to  980 mL with ddH<sub>2</sub>O
- Add  4 mL Phenol red indicator solution
- Dissolve the Guanidine hydrochloride by mixing on a magnetic stirrer
- Adjust to  6 with HCl
- Adjust volume to  1 L with ddH<sub>2</sub>O
- Sterilize by filtering and store at  Room temperature


 1 L TE minimum buffer  8




- Add  10 mL Tris stock solution  8 to a beaker
- Add  200 µL EDTA stock solution  8
- Adjust volume to  1 L with ddH<sub>2</sub>O
- Sterilize by filtering and store at  Room temperature




 100 mL silica beads working solution





- Add  5 mL SeraSil-Mag 400 beads to a clean  125 mL Nalgene bottle
- Add  25 mL TE minimum buffer
- Shake the bottle to wash the beads
- Place the bottle on a large magnet for  00:05:00 to pellet the beads
- Discard the supernatant

- Add  25 mL TE minimum buffer
- Shake the bottle to wash the beads
- Place the bottle on a large magnet for  00:05:00 to pellet the beads
- Discard the supernatant
- Add  100 mL TE minimum buffer
- Store at  Room temperature

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

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

 1 L elution buffer (  10 millimolar (mM) Tris )  8.5

- Add  10 mL Tris stock solution  8.5 to a beaker
- Adjust volume to  1 L with ddH<sub>2</sub>O
- Sterilize by filtering and store at  Room temperature

## Troubleshooting

## Safety warnings

- ! Buffers containing guanidine produce highly reactive compounds when mixed with bleach. Don't mix the extraction waste with bleach or solutions that contain bleach.  
Reagents are potentially damaging to the environment. Dispose waste as mandated.

## Before start




Make sure all buffers are prepared before starting.


1 To clear the lysates  11.000 x g, 20°C, 00:03:00

3m


## Bead-based protocol

2m

2 Prepare  240 µL GuHCl binding buffer and  20 µL silica beads working solution per sample in a  1.2 mL square well plate

3 Add  100 µL of the cleared lysate


### Note

The amount of lysate used in this protocol is flexible as long as it fits the plate used in the protocol. If the amount is to be changed the amount of binding buffer has to be adjusted accordingly as well to maintain a constant ratio of **lysate volume +  20 µL beads** to **binding buffer**.

**The binding buffer volume can be calculated as follows:**

**binding buffer volume = 2 x (lysate volume +  20 µL beads )**

4  700 rpm, Room temperature , 00:05:00 to bind the DNA to the beads


5 Place the plate on a magnet to pellet the beads for  00:02:00


2m

### Note


Depending on the magnet and volume used separation times may vary and have to be adjusted accordingly.

6 Discard the supernatant by pipetting

7 Add  100 µL of wash buffer to each sample


8  1000 rpm, Room temperature , 00:01:00 to wash excess salt off the beads




9 Place the plate on a magnet to pellet the beads for  00:01:00

1m

10 Discard the supernatant by pipetting

11  go to step #7 and repeat once for a total of 2 washes


12 Incubate the plate at  50 °C to dry off residuals of ethanol

13 Add  100 µL elution buffer to each sample



14  1000 rpm, Room temperature , 00:05:00 to elute the DNA from the beads

#### Note


Elution at  50 °C or with pre-warmed elution buffer may increase the yield.

15 Place the plate on a magnet to pellet the beads for  00:02:00

2m

16 Transfer  95 µL of the DNA to a new PCR plate. Store at  -20 °C



#### Note

Leaving  5 µL of elution buffer is recommended to avoid carry-over of beads.

## Spin column protocol (centrifugation)


1m



17 Combine  400  $\mu$ L GuHCl binding buffer with  200  $\mu$ L of the cleared lysate , vortex shortly

Note



The amount of lysate used in this protocol is flexible. The ratio of GuHCl binding buffer to lysate should remain 2:1.

18 Load all of the volume on a silica spin column and  11.000 x g, Room temperature, 00:01:00 to bind the DNA, discard the flow-through

1m

Note


If the binding buffer - lysate mixture exceeds the bed volume of the spin column it has to be loaded as often as needed to pass the complete volume through the spin column.


19 Add  600  $\mu$ L of wash buffer to the spin column and  11.000 x g, Room temperature, 00:00:30 , discard the flow-through

30s


Note


The amount of wash buffer should be adjusted to the maximum volume that has been loaded on the column to bind the DNA to remove all remaining traces of salts.



20  go to step #19 and repeat for a total of 2 washes

21  11.000 x g, Room temperature, 00:01:00 to dry the silica membrane

1m



22 Discard the collection tube and place the spin column in a clean  1.5 mL microcentrifuge tube

23 Add  100  $\mu\text{L}$  of elution buffer directly to the silica membrane



24 Incubate for  00:03:00 at  Room temperature 3m

Note

Yield might be increased by using elution buffer pre-warmed to  50  $^{\circ}\text{C}$


25  11.000 x g, Room temperature, 00:01:00 to elute the DNA. Discard the spin column, and store the eluted DNA at  -20  $^{\circ}\text{C}$  1m

### Spin column protocol (vacuum manifold) 1m

26 Combine  400  $\mu\text{L}$  GuHCl binding buffer with  200  $\mu\text{L}$  of the cleared lysate , vortex shortly



Note

The amount of lysate used in this protocol is flexible. The ratio of GuHCl binding buffer to lysate should remain 2:1.

27 Load all of the volume on a silica spin column or 96-well filter plate placed in a vacuum manifold. Apply vacuum until all of the volume has passed the column (  00:02:00 ). Release the vacuum 2m

Note


If the binding buffer - lysate mixture exceeds the bed volume of the spin column or filter plate it has to be loaded as often as needed to pass the complete volume through the spin column or filter plate. Times for application of vacuum may vary depending on the pump used. If a well clogs completely, carefully clean the membrane with a sterile pipette tip without piercing it.

- 28 Add  600  $\mu$ L of wash buffer to the spin column or filter plate. Apply vacuum until all of the buffer has passed the column (  00:01:00 ). Release the vacuum

1m

Note



The amount of wash buffer should be adjusted to the maximum volume that has been loaded on the column to bind the DNA to remove all remaining traces of salts.

- 29  [go to step #28](#) and repeat for a total of 2 washes

- 30 Apply vacuum for  00:10:00 to completely dry the silica membrane

10m

Note




More time might be needed if a weaker pump is used. If traces of wash buffer remain on the membrane it should be dried at  50 °C for  00:05:00 on a heat block stacked inside of a 1.2 mL storage plate.

- 30.1 For spin columns:  
 [go to step #23](#) and follow the protocol for centrifugation

- 30.2 For 96-well filter plates:  
 Place a suitable collection plate in the vacuum manifold

#### Note

Depending on the elution volume different collection plates may be suitable. For large volumes a storage plate (1.2 mL or 2.2 mL) is recommended. For smaller volumes a 96-well PCR plate or a U-bottom assay plate is recommended.

30.3 Add  100  $\mu\text{L}$  of elution buffer directly to the silica membrane. Apply vacuum until all of the elution buffer has passed the column (  00:01:00 ). Store eluted DNA at  -20  $^{\circ}\text{C}$

1m

#### Note

Yield might be increased by using elution buffer pre-warmed to  50  $^{\circ}\text{C}$