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Historical Samples Extraction

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

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Disclaimer

Our protocols are constantly evolving and old versions will be deleted.
The documents here are not intended to be cited in publications

Abstract

Extraction protocol for historical samples. Requires overnight incubation and rotary mixer.

Attachments

sds_EDTA.pdf 56KB
sds_S3379_sarkosyl.p... 62KB
sds_m6250_2mercaptoe... 179KB
sds_600201m_trisHCL... 26KB
sds_GuanidineThiocya... 56KB
museum_sample_extrac.. 45KB

Guidelines

Important Safety Note: Complete extraction steps in a **fume hood** and be very careful about any skin contact. Extraction buffer contains hazardous chemicals with inhalation hazards. Separate hazardous lab waste is needed for all waste products. See **Safety Warnings** below for more information.

Materials

Silica Magnetic Beads
guanidine isothiocyanate
1M Tris-HCl ph 7.5
0.2M EDTA
20% Sarkosyl
B-mercaptoethanol
PE Buffer
ddH2O

Safety warnings

Hazardous Chemicals Used (if applicable; SDS attached in **Description**):

- **Guanidine isothiocyanate**: causes severe skin burns and eye damage, harmful if swallowed, in contact with skin, or inhaled. If exposure occurs, immediately wash face, hands, and any exposed skin. Use in fumehood or well-ventilated area.
- **0.2M-0.5M EDTA**: harmful if inhaled, best to use in fumehood or well-ventilated area.
- **B-mercaptoethanol**: acute toxicity and highly flammable. Avoid skin exposure or inhalation. Use in fumehood.

Safety Note

1 Complete extraction steps in a **fume hood** and be very careful about any skin contact. Extraction buffer contains hazardous chemicals. Separate hazardous lab waste is needed for all waste products. See **Guidelines & Warnings** for more information.

Extraction Buffer Preparation

2 In a **50 mL** conical:

- **50 g** guanidine isothiocyanate
- **5.3 mL** 1M Tris-HCl pH 7.5
- **5.5 mL** 0.2M EDTA
- **10.6 mL** 20% Sarkosyl
- **1 mL** B-mercaptoethanol

Add **50 mL** of **ddH2O.**

Note

*If using **0.5M EDTA**, use **2.2 mL** instead (remaining volume will be water) to dilute to 0.2M EDTA.*

3 **ALTERNATIVE Buffer Prep SCALED** for Fewer Samples:

Volumes below are scaled to number of samples but you must make batches for at least 10--choose a total volume that is easy to check by eye for addition of ddH2O (amount for 1 sample: 0.2g guanidine isothiocyanate, 21.2uL 1M Tris-HCl, 22uL 0.2M EDTA, 42.4uL Sarkosyl, 4uL B-mercaptoethanol; bring volume up to 200uL).

- 0.2 g **guanidine isothiocyanate** *(for 10 samples: 2g)*
- 21.2 µL **1M Tris-HCl pH 7.5** *(for 10: 212uL)*
- 22 µL **0.2M EDTA** or 8.8 µL **0.5M EDTA** *(for 10: 220uL)*
- 42.4 µL **20% Sarkosyl** *(for 10: 424uL)*
- 4 µL **B-mercaptoethanol** *(for 10: 40uL)*

Add 200 µL **ddH2O** *(for 10: 2mL)*

Day 1 - Incubation

4 **Important Safety Note**: Buffer mixture is flammable. Use caution if sanitizing with flame between specimens.

Note

Recommend prepping tissues in 1.5mL tubes first on separate lab bench, then transfer to fume hood and add extraction buffer. Work quickly to avoid further tissue degradation.

5 For each sample: place tissue in a 1.5 mL eppendorf tube with **200 uL** of **extraction buffer.**

6 Incubate on hot plate at 55 °C Overnight .

Day 2 - Silica Bead Preparation

3m

7

Note

Note: Sillica Bead Preparation does not need to be performed in fume hood. Can prep at NGS lab bench instead.

Pipette or vortex **G-Biosciences Silica Magnetic Beads** thoroughly to re-suspend.

8 Transfer 20 µL of beads into a clean, labelled 1.5 mL eppendorf tube.

Note: This volume is scaled (amount for a single sample: 20uL).

Note

Up to 15 samples-worth of beads can be prepped in a single 1.5mL tube. However, more beads take longer to dry, etc. Consider prepping each tube individually for small numbers of samples.

If scaled to 8 samples: Use 160 uL of beads.

9 Place the tube on the **magnetic stand** for 30 - 60 seconds until clear: 00:00:30 - 00:01:00 . 1m 30s
Use a pipette to discard the supernatant.

10 Remove the tube from the magnetic stand. Add 100 µL **ddH20** or **Elution Buffer** (do not use Elution Buffer for museum samples).
Resuspend the beads by pipetting or vortexing.
Note: This volume is scaled (amount for a single sample: 100uL)

Note

Elution buffer (if using): 10mM Tris-HCl, 1mM EDTA, pH 8.0

If scaled to 8 samples: Use 800 uL ddH20.

11 Place the tube on the **magnetic stand** for 30 - 60 seconds until clear: 00:00:30 - 00:01:00 . 1m 30s
Use a pipette to discard the supernatant.

12 Repeat steps 10-11 twice: go to step #10

13 Remove tube from the magnetic stand. Add 20 µL **ddH20**.
Note: This volume is scaled (amount for a single sample: 20uL).

If scaled to 8 samples: Use 160 uL ddH20.

14 Pipette to resuspend. If applicable, aliquot bead mixture into number of tubes equal to number of samples you are extracting (*number you scaled this protocol by*). Each should have ~ 26.3uL if aliquotting from larger volume.

If scaled to 8 samples: Should have 8 tubes with ~20 uL of bead mixture in each.

Day 2 - Extraction 1h 57m

15 *Reminder: Complete extraction steps that involve lysate in fume hood.* 2m

If any solid debris remains, centrifuge extraction 2 minutes: 12000 rpm, 00:02:00 .

16 Add extraction lysate (liquid only, should be ~200uL) to each tube with 20uL of silica beads mixture. Discard any debris left over from centrifugation.

17 Add **200 uL** of **100% Ethanol** to lysate + beads. Gently mix and incubate in a **rotary mixer** for 15 minutes: 00:15:00 . 15m

18 Place tubes on **magnetic stand** for approximately 5 minutes, until clear: 00:05:00 . 5m
Use a pipette to discard the supernatant.

19 Pipette **200 uL** of **PE Buffer** (Qiagen) and incubate in a **rotary mixer** for 10 minutes: 00:10:00 . 10m

20 Repeat steps 18 - 19 twice: go to step #18 . When ready to place on magnet stand for the final time (*moving on to step 21*), spin tubes down briefly (mini centrifuge) to get beads out of cap.

21 Keeping tubes on the magnetic stand, allow beads to **air dry** for 20 - 45 minutes: 00:30:00 - 00:45:00 . 1h 15m

22 Remove tubes from the magnet and elute beads in **20-50 uL** of **ddH2O**. Use pipette to gently mix and use the elution H2O to encourage beads off the side of the tube. Incubate at 55 °C for 10 minutes: 00:10:00 at 55 °C . 10m

23 If needed, briefly spin tubes down (mini centrifuge) to get liquid out of caps.
Place tubes on **magnetic stand**. Wait until liquid is clear.
Use a pipette to move supernatant to a clean/new labeled tube, and discard the beads.