Jan 30, 2026 Version 1

Binding buffer D (5M40) V.1

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlk8nnwl5r/v1

Anna Schmidt[1], Anya Patova[1], Elena Essel[1]

[1]Max Planck Institute for Evolutionary Anthropology

MPI EVA Ancient DNA C...

Ancient DNA Core Unit
MPI EVA

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.j8nlk8nnwl5r/v1

External link: https://www.pnas.org/doi/full/10.1073/pnas.1314445110, https://www.nature.com/articles/s41596-018-0050-5

Document Citation: Anna Schmidt, Anya Patova, Elena Essel 2026. Binding buffer D (5M40). **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlk8nnwl5r/v1

Manuscript citation:

Dabney, J., Knapp, M., Glocke, I., Gansauge, M. T., Weihmann, A., Nickel, B., ... & Meyer, M. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. Proceedings of the National Academy of Sciences, 110(39), 15758-15763.

Rohland, N., Glocke, I., Ayinuer-Petri, A., & Meyer, M. (2018). Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. Nature Protocols, 13, 2447-2461.

Created: July 24, 2024

Last Modified: January 30, 2026

Document Integer ID: 103973

Keywords: ancient dna extraction, binding buffer, degraded dna, sediment dna, dna, ancient bone, dna purification, ultrashort dna fragment, ancient dna core unit of the mpi, ancient dna core unit, automated dna purification, ancient dna core, purification, propanol, silica

Funders Acknowledgements:
Max Planck Society
Grant ID: -

Abstract

Binding buffer D (5 M guanidine hydrochloride, 40% (vol/vol) 2-propanol, 0.12 M sodium acetate and 0.05% (vol/vol) Tween-20) is used for silica based manual and automated DNA purification by the Ancient DNA Core Unit of the MPI-EVA.

Note

This document describes the preparation of 500 ml Binding buffer D used in manual and automated silica based DNA purification.

Materials

	Reagent/consumable	Supplier	Catalogue number
	Reagents		
	Guanidine hydrochloride (GuHCl)	Sigma Aldrich	G3272-1KG
	Water	Sigma Aldrich/Merck	1153332500
	2-Propanol for analysis	Sigma Aldrich	1096342511
	Tween-20	Thermo Fisher Scientific	11417160
	Consumables		
	50 ml serological pipet	Corning BV	357550
	Square media bottle 500 ml	VWR	391-0630
	Aluminum foil	Roth	AA76.1
	Bottle top filter, 500 ml	Corning BV	430513
	Disposable weighing pan ROTILABO® blue, antistatic	Roth	2159.2
	Parafilm	Thermo Fisher Scientific	11762644

Safety information

[CAUTION: GUANIDINE HYDROCHLORIDE]

Guanidine Hydrochloride is harmful if swallowed, inhaled, or absorbed through the skin. It can cause severe irritation to the eyes, skin, and respiratory tract. Always wear appropriate personal protective equipment, including gloves, face shield, and full body suits. Use a fume hood to avoid inhalation. In case of contact follow the first aid measures described in the data safety sheet. Seek medical attention if symptoms persist. Pregnant or breastfeeding individuals should avoid handling this substance.

Always refer to the Material Safety Data Sheet (MSDS) attached for detailed information.

Equipment

- Serological pipette controller (e.g. battery-powered pipetting aid ROTILABO, cat. no. TC16.1)
- Laboratory balance (e.g. Mettler Toledo Balance, type XPR603SN)
- Vacuum pump (e.g. KNF Vacuum pump LABOPORT, type N 938.50 KT.18)
- Glass funnel (decontaminated*)
- 500 ml quartz glas bottle with plastic screw cap (custom produced by "Quarzglas Komponenten und Service", decontaminated*)
- Microwave (e.g. microwave oven, neoLab Migge Laborbedarf, cat. no. 800062)

*See documents in the Appendix for decontamination instructions.

Protocol

	Reagent	Amount	Final concentration
	GuHCl	238.8 g	5 M
	water	fill up to 300 ml	
	2-Propanol for analysis	fill up to 500 ml	40 %
	Tween-20	0.25 ml	0.05 %

1. Obtain the required amount of GuHCl salt by pouring the salt onto a weighing pan and measuring it on a laboratory balance. Transfer the GuHCl into a 500 ml square bottle using a glass funnel.

2. Fill the bottle with water up to 300 ml. Shake the bottle until the salt is dissolved. If necessary, briefly heat the mixture in a microwave until the salt is dissolved completely.

Note

[Note]

It is acceptable to use the scale of the bottle to fill up to 300 ml with water.

Note

[Caution]

Microwave 2-3 min at maximum power. Open microwave every ~30 sec and examine buffer to avoid overheating. Keep the lid loose at the bottle while microwaving.

3. Fill up to 500 ml with 2-Propanol.

Note

[Note]

It is acceptable to use the scale of the bottle to fill up to 500 ml with 2-Propanol.

4. Add 0.25 ml Tween-20, close the bottle properly and mix the buffer by shaking.

5. Filter the buffer through a bottle top filter into a quartz glass bottle using a vacuum pump.

Note

[Note]

If the filtering is not working properly, ensure that all connections are tight. If necessary freshly wrap the connections with Parafilm to ensure that they are completely tight.

Note

[Note]

The glass bottle needs to be placed in a stable position to avoid dropping and spilling liquids.

6. Decontaminate the buffer using UV treatment. Instructions for UV-decontamination are provided in the Appendix.

Note

[Note]

Since the salt leads to corrosion of metal surfaces only use the cross linker labeled "for Binding Buffer only" and clean it properly after use by wiping with 70 % ethanol.

7. Refill the buffer to a fresh 500 ml squared bottle.

Note

[Labeling]

Use the corresponding pre-printed labels to identify the bottle(s). Fill in the batch ID, date, the initials of the person who prepared the buffer and mark the buffer as UV-decontaminated.

CAUTION: Every single bottle prepared on the same day gets a new batch ID. Name the batches with Roman numerals (e.g. batch I, batch II, etc.).

Note

[Documentation]

Note the lot numbers, date and initials written on the reagents used for buffer preparation in the respective fields in Labfolder.

8. For automated DNA extraction divide the buffer into 3 × 157.5 ml using serological glass pipettes and refill to fresh 250 ml squared bottles. Discard the leftover liquid.

Note

[Note]

For automated DNA extraction bring the properly labeled bottles to bring the bottle to the clean room automation lab using the transfer box.

9. Store the buffer at room temperature until used. Shelf life is at least one year from preparation.

Note

[Note]

Visually check the buffer for small crystals before usage. If crystals have formed microwave the buffer briefly to dissolve them.

Appendix

Document

NAME

Bleaching and UV decontamination of materials

CREATED BY

Elena Essel

Preview

Document

NAME

UV decontamination of reagents/buffers

CREATED BY

Elena Essel

Preview

MSDS_Guanidine_hydrochloride.pdf 366.1KB