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1xBWT buffer V.1

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Abstract

Protocol for the preparation of 1xBWT buffer (Maricic et al. 2010), which is used in various protocols of the Ancient DNA Core Unit of the MPI-EVA, especially those involving streptavidin bead binding and washing.

References

Maricic, T., Whitten, M., Pääbo, S. (2010) Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. *PLoS One*, 16;5(11):e14004. doi: 10.1371/journal.pone.0014004

Troubleshooting

Note

This protocol describes the preparation of 500 ml buffer.

Materials

	Reagent/consumable	Supplier	Catalogue number
	Reagents		
	Water	Sigma Aldrich/Merck	1153332500
	5 M NaCl	Sigma Aldrich/Merck	S5150-1L
	1 M Tris-HCl, pH 8.0	AppliChem	A4577,1000
	0.5 M EDTA, pH 8.0	AppliChem	A4892,1000
	Tween-20	Thermo Fisher Scientific	11417160
	Consumables		
	Square media bottle 500 ml	VWR	391-0630
	50 ml serological pipette	Corning BV	357550
	5 ml serological pipette	Corning BV	357543

Equipment

- Serological pipette controller (e.g. battery-powered pipetting aid ROTILABO, cat. no. TC16.1)

Protocol

1. Prepare the buffer in a 500 ml square media bottle by adding the following reagents. Use the glass pipette for transfer of large volumes (> 1 ml). Mix reagents by shaking the bottle.

	Reagent	Volume	Final concentration in reaction
	Water	393.75 mL	



	Reagent	Volume	Final concentration in reaction
	5 M NaCl	100 mL	1 M
	1 M Tris-HCl, pH 8.0	5 mL	10 mM
	0.5 M EDTA, pH 8.0	1 mL	1 mM
	100% Tween-20	0.25 mL	0.05%
	<i>sum</i>	<i>500 mL</i>	

Note

[Labeling]

Label the bottle with the buffer name, batch ID, date and the initials of the person who prepared the buffer.

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

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

Note

[Documentation]

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