Instructions for Isohelix *Xtreme* DNA Kit: XME-5/50

Product Details

The Isohelix *Xtreme* DNA kit a silica membrane based spin column DNA purification kit designed to isolate highly purified DNA from buccal swabs with minimal losses. A260/280 ratios are typically >1.8 and A260/230 ratios are typically >1. 5

Key Benefits

- ✓ Very high purity DNA
- ✓ Removes PCR inhibitors
- ✓ Optimised for buccal DNA
- ✓ Protocol integrated to Isohelix swabs
- ✓ Manual or high throughput formats
- ✓ No solvent based chemicals

Kit Contents

	Isohelix *Xtreme* DNA Kit		
Catalogue No.	XME-50	XME-5	Storage temperature
Number of preps	50	5	
Contents:			
Solution LYS (Lysis buffer)	27ml	2.7ml	Room temperature
Solution CB (Column Binding buffer)	40ml	4ml	Room temperature
Solution WB (Wash buffer)	15ml *2	1.5ml*1	Room temperature
Solution EB (Elution buffer)	6ml	0.6ml	Room temperature
Proteinase K	2 x 11mg*4	2.2mg*3	4°C after reconstitution
Xtreme DNA Columns	50 pieces	5 pieces	Room temperature
Collection Tube	100 pieces	10 pieces	Room temperature
Protocol			

*1 Add 6 ml of 98-100% ethanol into solution WB before first use, tighten the cap securely to prevent ethanol evaporation.
*2 Add 60ml of 98-100% ethanol into solution WB before first use, tighten the cap securely to prevent ethanol evaporation.
*3 Reconstitute vial with 110µl ddH_2O before first use, store the solution at 4°C after reconstitution.
*4 Reconstitute each vial with 550µl ddH_2O before first use, store the solution at 4°C after reconstitution.

Storage

Isohelix *Xtreme* DNA Kits are shipped at ambient temperature.
Please note that on arrival the kit components should be stored according to the table above.
The kits are stable up to the expiry date if stored as instructed. See box label for expiry date.

Equipment and reagents to be supplied by user

- Water bath or heating block at 60°C and 70°C
- Pipettes with disposable tips
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
- 1.5ml microcentrifuge tubes and 5ml or 10ml round bottom tubes
- Vortexer
- Ethanol
- Sterile ddH_2O

Before Starting

1. Prepare a waterbath at 60°C.
2. Reconstitute the Proteinase K by adding appropriate amount of sterile water as shown above.
3. Add the appropriate amount of 98-100% ethanol to the WB bottle before use as shown above.

Technical Assistance

If you have any questions regarding the use of this kit or other Isohelix products please contact us by email at info@isohelix.com or for further information visit the website at www.isohelix.com

Safety and Use of the Isohelix *Xtreme* DNA kits

Buffers in the *Xtreme* DNA kits contain irritants so appropriate safety equipment such as gloves, laboratory coats and eye protection should be worn. The kits are intended for use by qualified professionals trained in potential laboratory hazards and good laboratory practise. If direct information is not available on any of our compounds this should not be interpreted as an indication of product safety.

This kit has been designed for research use only

Xtreme DNA Kit Protocol for XME-5/50

1. Place the swab head into a suitable tube. If using Isohelix SK-1 or SK-2 swabs, use the tube provided*.
2. Add 500µl LYS lysis buffer and vortex to cover the swab head.
3. Add 20µl Proteinase K solution, mix immediately by vortexing.
4. Incubate at 60^{0}C for a **minimum** of 10 minutes or up to 60 minutes to lyse the sample.

*When using SK-2 tubes, after lysis transfer the liquid to a clean 5ml tube before proceeding with step 5

5. Add 750µl CB buffer, mix by vortexing thoroughly for 30 seconds.
6. Preheat the EB buffer at 70^{0}C (100µl per sample).
7. Add 1.25ml ethanol to the sample and vortex to mix.
8. Place an *Xtreme* DNA column onto a collection tube. Pipette 700µl of the sample into the column without touching the rim. Centrifuge at maximum speed (13.4K rpm, 12,000 x g) for 1 minute. Discard the flow-through.
9. Repeat step 8 until all the sample has been loaded onto the column.
10. Wash the column by adding 750µl solution WB. Centrifuge at maximum speed (13.4K rpm, 12,000 x g) for 1 minute. Discard the flow-through.
11. Repeat the wash step by adding a further 750µl solution WB. Centrifuge at maximum speed (13.4K rpm, 12,000 x g) for 1 minute. Discard the flow-through.
12. Place the column in a clean collection tube and centrifuge at maximum speed (13.4K rpm, 12,000 x g) for 3 minutes to remove all traces of ethanol.
13. Place the column in a clean 1.5ml microcentrifuge tube. Add 100µl EB buffer pre-heated at 70^{0}C to the centre of the membrane.
14. Stand the column for 3 minutes then centrifuge at maximum speed (13.4K rpm, 12,000g) for 1 minute to elute the DNA.
15. Store the eluted DNA at –20^{0}C.

Typical A260/280 ratios for the eluted DNA are >1.8 and A260/230 ratios are >1.0