Isohelix *Xtreme* DNA Kit: XME-5/50 for use with GeneFiX Stabilized Saliva

Product Details

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

Key Benefits

- ✓ Very high purity DNA
- ✓ Removes PCR inhibitors
- ✓ Optimised for saliva DNA
- ✓ Protocol integrated to Isohelix GeneFiX
- ✓ Manual or high throughput formats
- ✓ Scalable to sample volume

Kit Contents

Isohelix *Xtreme* DNA Kit			
Catalogue No.	XME-50	XME-5	Storage temperature
Number of preps	**50 x 0.5ml**	**5 x 0.5ml**	
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	1 x 22mg*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			

Note: Solution LYS is not required for GeneFiX Saliva DNA Isolation.

*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 1.1ml 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 or 2ml microcentrifuge tubes and 5ml / 10ml or 15ml capped tubes
- Vortexer
- Ethanol
- Sterile ddH_2O
- Optional: Vacuum manifold suitable for spin columns

Before Starting

1. Prepare a water bath 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 practice. 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 XME-5/50: Protocol for DNA Isolation from Stabilized Saliva

Part A – DNA stabilisation

1. Use GeneFiX GFX-01 /GFX-02 or GFXA-01 to collect 1ml or 2ml saliva according to the collection instructions.
2. Seal the tube then vortex briefly or invert to mix.

At this point the DNA is stabilised. You may continue with the DNA isolation or store the stabilised saliva in the GeneFiX tube at room temperature for at least 5 years.

Part B – DNA Isolation from 0.5ml GeneFiX Saliva

3. Vortex the GeneFiX tube briefly and remove 0.5ml of the GeneFiX stabilised saliva into a clean capped tube with a minimum volume of 5ml. Add 20µl Proteinase K solution, mix immediately by vortexing. **See note 1 below.**
4. Incubate at 60^0C for 1 hour, or a minimum of 30 minutes to lyse the sample.
5. Add 750µl CB buffer, mix by vortexing thoroughly for 30 seconds.
6. Preheat the EB buffer at 70^0C (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. **See note 2 below.**
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 70^0C 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 -20^0C.

Typical A260/280 ratios for the eluted DNA are 1.8 and A260/230 ratios are >1.5

Note 1 for step 3: The sample volume can be scaled upwards as shown in the table below.

GeneFiX Sample Volume	Volume PK added	Tube volume required	Volume CB added	Volume ethanol added	Volume EB used	**Number of preps per XME-50 kit**
0.5ml	20µl	5ml	750µl	1.25ml	100µl	**50**
1ml	20µl	10ml	1.5ml	2.5ml	100µl	**25**
2ml	20µl	15ml	3ml	5ml	200µl	**12**

Note 2 for steps 9 to 11: Use of a vacuum manifold for loading the sample onto the Xtreme DNA columns, and for performing the 2 wash steps is a recommended option for quicker sample throughput.