High Throughput 96 Deep Well Plate Method

Instructions for Isohelix GeneFix™ Saliva-Prep 2 DNA Kit: GSPN-96/HT

Product Details

The Isohelix GeneFiX™ GFX-02 Saliva collectors are designed to collect a 2ml saliva sample into 2ml lysis buffer pre-filled into the 10ml collection tube, giving a total volume of 4ml, or for the GFX-01 Saliva collectors to collect a 1ml saliva sample into 1ml lysis buffer.
With the Assisted Collection Kit, the volume of saliva collected on 2 sponges is released into 1ml lysis buffer pre-filled into the 10ml collection tube. The GeneFiX™ Saliva-Prep DNA kit is designed to process either the whole sample in one step or smaller aliquots of the stabilised sample.

Key Benefits

- ✓ Integrated to Isohelix GeneFiX™ collectors
- ✓ Optimised for saliva DNA
- ✓ High yield and purity
- ✓ Manual or high throughput formats
- ✓ Fast handling times
- ✓ No columns or filtration
- ✓ No solvent based chemicals
- ✓ Less consumables wastage

Kit Contents

Isohelix GeneFiX™ Saliva-Prep 2 DNA Kit for 96 x 0.5ml GeneFiX™ saliva samples		
Catalogue No.	**GSPN-96/HT**	**Storage temperature**
Number of GFX samples	96	
Contents:		
Solution SPN	100ml	Room temperature
Solution TE	20ml	Room temperature
Solution SLS	20ml	Room temperature
DNA Rehydration buffer	10ml	Room temperature
Proteinase K	1 x 22mg *1	4^0C after reconstitution
Protocol		

Reconstitute the vial with **1.1ml** sterile ddH_2O before first use, store at 4^0C after reconstitution.

Storage

Isohelix GeneFiX™ Saliva-Prep 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

- Waterbath or heating block at 60^0C
- Pipettes, stepper and multi-channel pipettes with disposable tips
- Centrifuge with rotor and buckets suitable for 96 well deep well plates, capable of a minimum of 3000 x g, ideally 4,200 x g.
- 1.6ml or 2.0ml capacity 96 well deep well plates with adhesive sheets or re-useable mats, capable of centrifugation at 4,200 x g.
- 1.5ml or 2ml microcentrifuge tubes
- Vortexer

Before Starting

1. Preheat waterbath or heating block to 60^0C.
2. Reconstitute the Proteinase K by adding the appropriate amount of sterile ddH_2O 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 GeneFiX™ Saliva DNA kits

Buffers in the GeneFiX™ 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

Protocol for high throughput isolation of 500µl GeneFiX™ samples using deep well plates

The GSPN-96/HT kit provides sufficient reagents to process 96 x 500µl stabilised GeneFiX™ saliva samples using the protocol below.

1. Vortex the GeneFiX™ saliva collection tube to mix well.
2. Remove 500µl sample to a 1.5ml or 2ml tube, add 5µl Proteinase K solution, vortex to mix then incubate at 60^{0}C for 1 hour (increase the incubation time to 2 hours if using a hot air oven).
   See alternative option for Proteinase K treating the whole 4ml GeneFiX™ sample below.
3. Place 500µl SPN buffer into each well of a deep well plate (well capacity 1.6ml or 2.0ml).
4. Add 500µl Proteinase K treated GeneFiX™ sample to each well containing SPN buffer and mix several times with a pipette tip.
5. Cover the plate with an adhesive seal or re-useable mat and centrifuge at room temperature for 20 minutes at 4,200 x g, or for 30 minutes at 3,000 x g.
6. Pour off the supernatant and place the plate upside down on absorbent paper until the plate is fully blotted dry.
7. Add 200µl TE to each well containing sample. Use a multi-channel pipette set at 100ul and pipette up and down several times to resuspend the pellets.
8. Cover the plate with an adhesive seal or re-useable mat and centrifuge at room temperature for 30 minutes at 4,200 x g or 40 minutes at 3,000 x g.
9. Using a clean pipette tip for each sample, carefully transfer 200µl supernatant to a fresh 96 well deep well plate, without disturbing the pellet.
10. Add 200µl SLS buffer to each well containing sample.
11. Add 400µl SPN buffer to each well containing sample and mix several times with a pipette tip using a multi-channel pipette.
12. Cover the plate with an adhesive seal or re-useable mat and centrifuge at room temperature for 20 minutes at 4,200 x g or for 30 minutes at 3,000 x g.
13. Pour off the supernatant and place the plate upside down on absorbent paper until the plate is fully blotted dry. **Note**: It is important that all the liquid has been removed at this point.
14. Add 50µl DNA rehydration buffer to each well, leave to stand for 5 minutes to allow the DNA to re-hydrate then mix several times using a multi-channel pipette set at 30µl. Cover with an adhesive seal or re-useable mat and spin for 5 minutes at 4,200 x g or for 10 minutes at 3,000 x g.
15. Using a clean pipette tip for each sample, carefully remove the supernatant either to a clean plate or a separate tube for analysis and storage. If the samples are being stored in a plate, cover the plate with an adhesive seal to prevent evaporation.

Alternative option for Proteinase K treating the whole 4ml GeneFiX™ sample:

If you prefer to Proteinase K treat the whole 4ml GeneFiX™ sample, add 40µl Proteinase K to the GeneFiX™ tube and incubate at 60C for 1 hour (increase the incubation time to 2 hours if using a hot air oven).
Add 500µl of the PK treated sample to a well containing 500µl SPN buffer in step 4.
The remainder of the PK treated GeneFiX™ sample is stable for long term storage at room temperature.
Additional Proteinase K can be purchased from Isohelix, Cat. No: PK/22 for 22mg lyophilised Proteinase K.