PLEASE NOTE NEW INSTRUCTIONS INCLUDING OPTIONAL STEPS FOR INCREASED PURITY

Instructions for Isohelix GeneFix™ Saliva-Prep 2 DNA Kit: GSPN- 50 / GSPN-12 / GSPN-2

Product Details

The Isohelix GeneFix™ Saliva collectors are designed to collect saliva samples into lysis buffer pre-filled into the collection tube, with the sample and buffer in equal volumes.
With the Assisted Collection Kit the volume of saliva collected on 2 sponges is released into 1ml lysis buffer pre-filled into the 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 according to the protocols shown following.

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 2ml or 1ml saliva samples				
Catalogue No.	**GSPN-50**	**GSPN-12**	**GSPN-2**	**Storage Temperature**
No. of GFX-02 samples	50	12	2	
No. of GFX-01 samples	100	24	4	
Contents:				
Proteinase K	2 x 22mg*	11mg*	2.2mg*	4^0C *
Solution SPN	2 x 120ml	58ml	9ml	Ambient
Solution TE	40ml	10ml	1.6ml	Ambient
Solution SLS	20ml	5ml	0.8ml	

* Reconstitute each vial with **1.1ml (22mg), 550µl (11mg) or 110µl (2.2mg)** 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 userBefore Starting

- Waterbath or heating block at 60^0C
- Pipettes with disposable tips
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
- Centrifuge with rotor for 15ml conical centrifuge tubes
- 15ml conical centrifuge tubes
- 2ml V bottom microcentrifuge tubes and 1.5ml microcentrifuge tubes
- Vortexer

1. Prepare waterbath or heating block at 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

Complete GFX-02 or *GFX-01* sample

Isolation Protocol (50:50 Saliva to Stabilization buffer)

1. Vortex the GeneFix™ saliva collection tube to mix well.
2. Add **40µl** /*20µl* Proteinase K solution, vortex to mix then incubate at 60^{0}C for 1 hour, or a minimum of 30 minutes.
3. Transfer the solution to a 15ml conical centrifuge tube. Add **4ml** / *2ml* SPN buffer, vortex well to mix thoroughly.
4. Centrifuge at 4.4K rpm/3,000 x g for 30 minutes. Pour off the supernatant then re-spin briefly.
5. Remove all remaining liquid with a pipette tip taking care not to disturb the DNA pellet. **Note** it is important to remove all the liquid.
6. Add **400µl** / *200µl* TE buffer to the tube, vortex well and leave at room temperature for at least 5 minutes for the DNA to re-hydrate.
7. Transfer the sample to a 2ml V bottom microcentrifuge tube and centrifuge at maximum speed, 13.4K rpm/12,000 x g for 15 minutes to remove any undissolved particulates, remove the supernatant to a clean 2ml tube being careful not to disturb the pellet.
8. The sample purity can now be assessed by nanodrop. If sample purity is not sufficient continue with the steps below.
9. Add **400µl** / *200µl* SLS buffer to the tube. Vortex to mix. Add **800µl** / *400µl* SPN buffer to the tube, vortex well to mix.
10. Centrifuge at 13.4K rpm/12,000 x g for 10 minutes. Pour off the supernatant, re-spin briefly and carefully remove the remaining liquid with a pipette tip. The pellet may not be visible at this point.
11. Add **400µl** / *200µl* TE buffer, vortex well and leave at room temperature for at least 5 minutes for the DNA to re-hydrate.
12. Store the DNA sample at 4^{0}C for short term storage or at -20^{0}C for long term storage.

1ml* GeneFix™ saliva sample

Microcentrifuge Isolation Protocol

1. Vortex the GeneFix™ saliva collection tube to mix. Remove 1ml sample into a 2ml microcentrifuge tube.
2. Add 10µl Proteinase K solution, vortex to mix then incubate at 60^{0}C for 1 hour, or a minimum of 30 minutes.
3. Add 1ml SPN buffer, vortex well to mix thoroughly.
4. Centrifuge at 13.4K rpm/12,000 x g for 10 minutes. Pour off the supernatant then re-spin briefly.
5. Remove all remaining liquid with a pipette tip taking care not to disturb the DNA pellet. **Note** it is important to remove all the liquid.
6. Add 100µl TE buffer to each tube, vortex well and leave at room temperature for at least 5 minutes for the DNA to re-hydrate.
7. Centrifuge the tube at maximum speed, 13.4K rpm/12,000 x g for 15 minutes to remove any undissolved particulates, remove the supernatant to a clean 1.5ml or 2ml tube being careful not to disturb the pellet.
8. The sample purity can now be assessed by nanodrop. If sample purity is not sufficient continue with the steps below.
9. Add 100µl SLS buffer to the tube. Vortex to mix. Add 200µl SPN buffer to the tube, vortex well to mix.
10. Centrifuge at 13.4K rpm/12,000 x g for 10 minutes. Pour off the supernatant, re-spin briefly and carefully remove the remaining liquid with a pipette tip. The pellet may not be visible at this point.
11. Add 100µl TE buffer, vortex well and leave at room temperature for at least 5 minutes for the DNA to re-hydrate.
12. Store the DNA sample at 4^{0}C for short term storage or at -20^{0}C for long term storage.

***For different volumes of sample, the reagent volumes can be calculated pro-rata for steps 1-5 and 11**

High Throughput:

Protocol for High Throughput Isolation of 500µl* GeneFix™ samples using deep well plates

The GSPN-50 kit provides sufficient reagents to process 100 stabilized saliva samples using the protocol below.
Requires

- 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.
- 1.2ml capacity 96 well deep well plates with adhesive sheets or re-useable mats, capable of withstanding centrifugation at 4,200 x g.

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 (minimum well capacity 1.2ml)
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 ambient 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 (minimum well capacity 1.2ml), 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, or by covering the plate with an adhesive seal or re-useable mat and vortexing the plate.
12. Cover the plate and centrifuge at room temperature for 20min at 4,200 x g or for 30min 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. If necessary, re-spin briefly to collect any remaining liquid and carefully remove with a pipette tip without disturbing the pellet.
14. Add 50µl DNA rehydration buffer to each well, mix several times using a multi-channel pipette to resuspend the pellet, stand for 5 minutes at room temperature to allow the DNA to fully re-hydrate then 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. 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.

DNA yields measured by Qubit assay are typically well in excess of 30µg from a 2ml saliva sample, A260/280 ratios for the final DNA sample are typically >1.75 and A260/230 >1.6

***For different volumes of sample, the reagent volumes can be calculated pro-rata for steps 1 to 7 and 16**

Assisted Collectors:

Isolation Protocol for 1ml GeneFix™ Assisted Collection saliva sample

(Equivalent to 2 sponges collected into 1ml lysis buffer)

1. Add 20µl Proteinase K solution to the GeneFix™ tube, vortex well to mix then incubate at 60^{0}C for 1 hour, or a minimum of 30 minutes.
2. Note the sample volume in the tube and add an equal volume of SPN buffer, vortex well to mix thoroughly.
3. Transfer the whole sample to a 15ml centrifuge tube and spin at 4.4K rpm/3,000 x g for 30 minutes. Pour off the supernatant then re-spin briefly.
4. Remove all remaining liquid with a pipette tip taking care not to disturb the DNA pellet. **Note** it is important to remove all the liquid.
5. Add 200µl TE buffer to the tube, vortex well and leave at room temperature for at least 5 minutes for the DNA to re-hydrate.
6. Transfer the sample to a 2ml V bottom microcentrifuge tube and centrifuge at maximum speed, 13.4K rpm/12,000 x g for 15 minutes to remove any undissolved particulates, remove supernatant to a clean 1.5ml or 2ml tube being careful not to disturb the pellet.
7. The sample purity can now be assessed by nanodrop. If sample purity is not sufficient continue with the steps below.
8. Add 200µl SLS buffer to the tube. Vortex to mix. Add 400µl SPN buffer to the tube, vortex well to mix.
9. Centrifuge at 13.4K rpm/12,000 x g for 10 minutes. Pour off the supernatant, re-spin briefly and carefully remove the remaining liquid with a pipette tip. The pellet may not be visible at this point.
10. Add 200µl TE buffer, vortex well and leave at room temperature for at least 5 minutes for the DNA to re-hydrate.
11. Store the DNA sample at 4^{0}C for short term storage or at -20^{0}C for long term storage.