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

## 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 according to the protocols shown on page 2.

## 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** |
| Number of GFX-02 samples | 50 | 12 | 2 | |
| Number of GFX-01 samples | 100 | 24 | 4 | |
| Contents: | | | | |
| Proteinase K | 2 x 22mg*1 | 11mg*2 | 2.2mg*3 | 4$^0$C after reconstitution |
| Solution SPN | 2 x 120ml | 58ml | 9ml | Room temperature |
| Solution TE | 40ml | 10ml | 1.6ml (Black cap) | Room temperature |
| Solution SLS | 20ml | 5ml | 0.8ml (Purple cap) | Room temperature |
| Protocol | | | | |

*1 Reconstitute each vial with **1.1ml** sterile dd$H_2O$ before first use, store at 4$^0$C after reconstitution.
*2 Reconstitute vial with **550µl** sterile dd$H_2O$ before first use, store at 4$^0$C after reconstitution.
*3 Reconstitute vial with **110µl** sterile dd$H_2O$ before first use, store at 4$^0$C 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$^0$C
- 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

## Before Starting

1. Prepare waterbath or heating block at 60$^0$C.
2. Reconstitute the Proteinase K by adding the appropriate amount of sterile dd$H_2O$ 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**

## Isolation Protocol for GFX-02 4ml GeneFix™ saliva sample (2ml saliva collected into 2ml lysis buffer)

1. Vortex the GeneFix™ saliva collection tube to mix well.
2. Add 40µl 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 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 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. Add 400µl SLS buffer to the tube. Vortex to mix. Add 800µl 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 400µl 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.

## Isolation Protocol for GFX-01 2ml GeneFix™ saliva sample (1ml saliva collected into 1ml lysis buffer)

1. Vortex the GeneFix™ saliva collection tube to mix well.
2. Add 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 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 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 1.5ml or 2ml tube being careful not to disturb the pellet.
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.

## Microcentrifuge Isolation Protocol for 1ml GeneFix™ saliva sample

1. Vortex the GeneFix™ saliva collection tube to mix well. 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. Add 100µl SLS buffer to the tube. Vortex to mix. Add 200µl 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 100µl 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.

## 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. Add 200µl SLS buffer to the tube. Vortex to mix. Add 400µl SPN buffer to the tube, vortex well to mix.
8. 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.
9. Add 200µl TE buffer, vortex well and leave at room temperature for at least 5 minutes for the DNA to re-hydrate.
10. Store the DNA sample at 4$^0$C for short term storage or at -20$^0$C for long term storage.

**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**