Instructions for Isohelix GeneFix™ Saliva-Mag DNA Kit: GSM-2/GSM-12/GSM-48

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-Mag 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-Mag DNA Kit for 2ml or 1ml saliva samples				
Catalogue No.	**GSM-2**	**GSM-12**	**GSM-48**	**Storage temperature**
Number of GFX-02 samples	2	12	48	
Contents:				
Proteinase K	2.2mg*1	11mg*2	2 x 22mg*3	4^0C after reconstitution
Binding Buffer	8ml	50ml	2 x 100ml	Room temperature
Magnetic Beads	80µl	480µl	2 x 1ml	Store at 4^0C
Wash Buffer	30ml*4	2 x 100ml*5	2 x 350ml*6	Room temperature
Elution Buffer	800µl	4.8ml	20ml	Room temperature
Protocol				

*1 Reconstitute vial with **220µl** sterile ddH_2O before first use, store at 4^0C after reconstitution.
*2 Reconstitute vial with **550µl** sterile ddH_2O before first use, store at 4^0C after reconstitution.
*3 Reconstitute vial with **1.1ml** sterile ddH_2O before first use, store at 4^0C after reconstitution.
*4 Add **21ml** 98-100% Ethanol to bottle before use.
*5 Add **70ml** 98-100% Ethanol to each bottle before use.
*6 Add **245ml** 98-100% Ethanol to each bottle before use.

Storage

Isohelix GeneFix™ Saliva-Mag 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 with disposable tips
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
- 2ml V bottom microcentrifuge tubes and 1.5ml microcentrifuge tubes
- Vortexer
- 98-100% Ethanol, Molecular Biology Grade

Before Starting

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.
3. Add the appropriate volume of 98-100% Ethanol (according to kit size) to the Wash Buffer bottle, invert to mix.
4. Vortex the tube of Magnetic Beads immediately prior to use, ensure the beads are all in suspension before adding to the sample.

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-Mag 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. Add 40µl Proteinase K solution, vortex to mix then incubate at 60^{0}C for 1 hour.
2. Add 4ml Binding Buffer and 40µl of fully resuspended Magnetic Beads to each tube, vortex well to mix thoroughly.
3. Stand at room temperature for 5 minutes to allow the DNA to bind to the magnetic beads.
4. Place the uncapped tube into the magnetic separator rack and stand for 3 minutes to allow the beads to separate.
5. With the tube still in the rack, carefully remove all of the liquid with a 10ml pipette, and discard.
6. Transfer the tube to a separate rack. Add 8ml wash buffer and resuspend the magnetic beads either using a 1ml pipette tip or replacing the lid and vortexing the tube until all of the beads have been resuspended.
7. Replace the uncapped tube into the magnetic separator rack and leave for 2 minutes.
8. With the tube still in the rack, carefully remove all of the liquid with a 10ml pipette, and discard.
9. Repeat the wash steps 7-9 twice more, using 4ml Wash Buffer then 2ml Wash Buffer.
10. After the last wash has been removed, leave the uncapped tube in the magnetic rack for 5 minutes then use a pipette tip to remove any remaining liquid.
11. Add 400µl Elution Buffer, vortex to resuspend the beads. Incubate at 60^{0}C for 2 to 5 minutes.
12. Replace the uncapped tube into the magnetic rack, leave for 3 minutes.
13. Using a pipette tip, carefully remove the eluate into a clean 2ml or 1.5ml microcentrifuge tube, taking care not to disturb the beads.
14. Centrifuge the sample for 5 minutes at 13,400 rpm/12,000 x g then carefully remove the supernatant to a clean 1.5ml or 2ml tube without disturbing any pellet.
15. Store the isolated DNA short term at 4^{0}C or long term at -20^{0}C or -80^{0}C.

Isolation Protocol for a 0.5ml aliquot from a GFX-02 4ml GeneFix™ saliva sample

1. Vortex the GeneFix™ saliva collection tube to mix well. Remove 0.5ml sample into a 2ml V bottom microcentrifuge tube.
2. Add 5µl Proteinase K solution, vortex to mix then incubate at 60^{0}C for 1 hour.
3. Add 0.5ml Binding Buffer and 5µl of fully resuspended Magnetic Beads to each tube, vortex well to mix thoroughly.
4. Stand at room temperature for 5 minutes to allow the DNA to bind to the magnetic beads.
5. Place the uncapped tube into the magnetic separator rack and stand for 3 minutes to allow the beads to separate.
6. With the tube still in the rack, carefully remove all of the liquid with a 1ml pipette tip, and discard.
7. Transfer the tube to a separate rack. Add 1ml wash buffer and carefully resuspend the magnetic beads either using a 1ml pipette tip or replacing the lid and vortexing the tube until all of the beads have been resuspended.
8. Replace the uncapped tube into the magnetic separator rack and leave for 2 minutes.
9. With the tube still in the rack, carefully remove all of the liquid with a 1ml pipette tip, and discard.
10. Repeat the wash steps 7-9 twice more, using 0.5ml Wash Buffer then 0.25ml Wash Buffer.
11. After the last wash has been removed, leave the uncapped tube in the magnetic rack for 5 minutes then use a pipette tip to remove any remaining liquid.
12. Add 50µl Elution Buffer, vortex to resuspend the beads. Incubate at 60^{0}C for 2 to 5 minutes.
13. Replace the uncapped tube into the magnetic rack, leave for 3 minutes.
14. Using a pipette tip, carefully remove the eluate into a clean 2ml or 1.5ml microcentrifuge tube, taking care not to disturb the beads.
15. Centrifuge the sample for 5 minutes at 13,400 rpm/12,000 x g then carefully remove the supernatant to a clean 1.5ml or 2ml tube without disturbing any pellet.
16. Store the isolated DNA short term at 4^{0}C or long term at -20^{0}C or -80^{0}C.

Notes: The magnetic separator rack can process 12 x 4ml GeneFix samples in the original samples tubes, or 6 x 0.5ml-1ml aliquots in 2ml V bottom microcentrifuge tubes.

The Proteinase K step can be performed on the whole 4ml sample as described in the first protocol, then an aliquot removed for isolation. The remainder of the sample will be stable long term at room temperature.

The method is scalable for any volume of sample from 0.5ml to the full 4ml sample. Use 10ul Proteinase K per ml sample, use a volume of Binding Buffer equal to the sample volume, and use 10µl Magnetic Beads per ml sample. For the 3 wash steps, the first Wash Buffer volume should be equal to the sample + Binding Buffer volume, the second wash step should use 50% of the first Wash Buffer volume, and the third wash step use 25% of the first wash Buffer volume. Elute using 1/10th sample volume of elution buffer.

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