

Instructions for Isohelix GeneFix™ Saliva DNA Mini Kit: GSS-50/GSS-5

Product Details

The Isohelix GeneFix™ Saliva DNA Mini kit is a silica membrane based spin column DNA purification kit, designed to isolate highly purified DNA from 0.5 -1ml saliva samples collected and stabilised within Isohelix GeneFix™ saliva collectors. DNA yields are typically in excess of 10µg, typical A260/280 ratios for the eluted DNA are >1.8 and A260/230 ratios are >1.5.

Key Benefits

- ✓ Integrated to Isohelix GeneFix™ collectors
- ✓ Optimised for saliva DNA
- ✓ Very high purity DNA
- ✓ Manual or high throughput formats
- ✓ Fast handling times
- ✓ Removes PCR inhibitors
- ✓ Recovery rates up to 80%
- ✓ No solvent based chemicals

Kit Contents

Isohelix GeneFix™ Saliva DNA Mini Kit for 0.5-1ml saliva samples			
Catalogue No.	GSS-50	GSS-5	Storage temperature
Contents:			
Proteinase K	22mg*1	2.2mg*2	4°C after reconstitution
Solution WB (Wash buffer)	15ml *3	1.5ml*4	Room temperature
Solution EB (Elution buffer)	6ml	0.6ml	Room temperature
GeneFix™ Mini Columns	50 pieces	5 pieces	Room temperature
Collection tubes	100 pieces	10 pieces	Room temperature
Protocol			

*1 Reconstitute with 1.1ml sterile ddH₂O before first use, store the solution at 4°C after reconstitution.

*2 Reconstitute with 110µl sterile ddH₂O before first use, store the solution at 4°C after reconstitution.

*3 Add 60ml of 98-100% ethanol into solution WB before first use, tighten the cap securely to prevent ethanol evaporation.

*4 Add 6ml of 98-100% ethanol into solution WB before first use, tighten the cap securely to prevent ethanol evaporation.

Storage

Isohelix GeneFix™ Saliva DNA Mini 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°C and 70°C
- Pipettes with disposable tips
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
- 5ml sealable tubes (or larger)
- 1.5ml microcentrifuge tubes
- Vortexer
- Ethanol
- Sterile ddH₂O

Before Starting

1. Prepare waterbaths or heating blocks at 60°C and 70°C.
2. Reconstitute the Proteinase K by adding appropriate amount of sterile ddH₂O as shown above.
3. Add the appropriate amount of 98-100% ethanol to the WB bottle before use as shown above.

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

GeneFix™ Saliva DNA Mini Kit for 0.5 – 1ml saliva samples

The Isoheli GeneFix™ 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. The GeneFix™ Saliva DNA Mini kit is designed to process up to 2ml of saliva/lysis buffer mix (1ml whole saliva). If you require to process the whole 4ml sample (2ml whole saliva) in one isolation procedure, the GeneFix™ Saliva DNA **MIDI** kit (Cat. No: GMS-48/GMS-12) should be used instead.

Note: The protocol below is for processing 2ml of the saliva/lysis buffer mix. If a smaller volume is being used, at **Step 4** the volume of ethanol added should be **equal** to the volume of saliva/lysis buffer being processed as per the table below.

Sample Volume	Volume of Proteinase K	Volume of ethanol
0.5ml	10µl	0.5ml
1ml	20µl	1ml
2ml	20µl	2ml

Isolation Protocol

- Vortex the GeneFix™ saliva collection tube to mix well. Remove 2ml solution into a (minimum volume) 5ml tube.
- Add 20µl Proteinase K solution, vortex to mix then incubate at 60°C for 1 hour.
- Preheat the EB buffer at 70°C (100µl/sample).
- Add 2ml ethanol to the sample from step 2 and vortex to mix* **See note and table above**
- Place a GeneFix™ Mini DNA column onto a collection tube. Pipette 700µl of the sample into the column without touching the rim. Centrifuge at high speed (13.4K rpm, 12,000 x g) for 1 minute. Discard the flow-through.
- Repeat step 5 until all the sample has been loaded onto the column.
- Wash the column by adding 750µl solution WB. Centrifuge at high speed (13.4K rpm, 12,000 x g) for 1 minute. Discard the flow-through.
- Repeat the wash step by adding a further 750µl solution WB. Centrifuge at high speed (13.4K rpm, 12,000 x g) for 1 minute. Discard the flow-through.
- Place the column in a clean collection tube and centrifuge at high speed (13.4K rpm, 12,000 x g) for 3 minutes to remove all traces of ethanol.
- Place the column onto a clean 1.5ml microcentrifuge tube. Add 100µl EB buffer pre-heated at 70°C to the centre of the membrane.
- Stand the column for 3 minutes then centrifuge at high speed (13.4K rpm, 12,000 x g) for 1 minute to elute the DNA.
- Store the eluted DNA at -20°C.

A260/280 ratios for the eluted DNA are typically >1.8, and A260/230 ratios are >1.5. DNA yields should be in the range of 5 to 10µg per ml saliva with DNA concentrations in excess of 50ng/µl.