Instructions for Isohelix GeneFix™ Saliva DNA Midi Kit: GMS-48/GMS-12/GMS-2

Product Details

The Isohelix GeneFix™ Saliva DNA Midi kit is a silica membrane based spin column DNA purification kit, designed to isolate highly purified DNA from 2ml saliva samples collected and stabilised with Isohelix GeneFix™ saliva collectors. DNA yields are typically well in excess of 20µg, 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 Midi Kit for 2ml saliva samples

Catalogue No.	GMS-48	GMS-12	GMS-2	Storage temperature
Contents:				
Proteinase K	2 x 22mg*1	11mg*2	2 x 2.2mg*3	4°C after reconstitution
Solution WB (Wash buffer)	4 x 15ml *4	15ml *4	2.5ml*5	Room temperature
Solution EB (Elution buffer)	4 x 6ml	6ml	0.6ml	Room temperature
GeneFix™ Midi Columns	48 pieces	12 pieces	2 pieces	Room temperature
15ml Collection tubes	96 pieces	24 pieces	4 pieces	Room temperature
Protocol				

*1 Reconstitute each vial with 1.1ml sterile ddH_2O before first use, store the solution at 4°C after reconstitution.
*2 Reconstitute with 550µl sterile ddH ddH_2O before first use, store the solution at 4°C after reconstitution.
*3 Reconstitute each vial with 110µl sterile ddH ddH_2O before first use, store the solution at 4°C after reconstitution.
*4 Add 60ml of 98-100% ethanol to each bottle of WB before first use, tighten the cap securely to prevent ethanol evaporation.
*5 Add 10ml of 98-100% ethanol to the bottle of WB before first use, tighten the cap securely to prevent ethanol evaporation.

Storage

Isohelix GeneFix™ Saliva DNA Midi 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 for 10ml/15ml tubes
- Waterbath or heating block at 70°C
- Pipettes with disposable tips
- Microcentrifuge (with rotor for 1.5 ml and 2 ml tubes)
- Centrifuge with rotor for 15ml conical centrifuge tubes
- 2ml microcentrifuge tubes
- Vortexer
- Ethanol, 3M Sodium Acetate pH5.2, PBS
- Sterile ddH_2O

Before Starting

1. Prepare waterbaths or heating blocks at 60°C and 70°C.
2. Reconstitute the Proteinase K by adding the appropriate amount of sterile ddH_2O 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 Midi Kit for 2ml saliva samples

The Isohelix GeneFix™ Saliva collectors are designed to collect a 2ml saliva sample into 2ml stabilisation buffer pre-filled into the 10ml collection tube. If the total volume in the collection tube is significantly different to 4ml, yields may be sub-optimal and optional step A or step B should be followed.

A. If the total volume in the collection tube is <3ml, make the volume up to 4ml with PBS.

B. If the total volume in the collection tube is >4.5ml, write the total volume on the collection tube and at step 4* add a volume of ethanol **equal** to that of the total sample volume (instead of the stated 4ml).

Isolation Protocol

1. Add 40µl Proteinase K solution, mix immediately by vortexing.
2. Incubate at 60^{0}C for 1 hour to lyse the sample.
3. Preheat the EB buffer at 70^{0}C (0.4ml/sample).
4. Add 4ml ethanol to the sample and vortex to mix* See optional step B
5. Place a GeneFix™ Midi DNA column onto a 15ml collection tube. Pipette 2ml of the sample into the column without touching the rim. Centrifuge at high speed (minimum 3500 x g) for 1 to 2 minutes. Discard the flow-through.
6. Repeat step 5 until all the sample has been loaded onto the column.
7. Wash the column by adding 2.5ml solution WB. Centrifuge at high speed (minimum 3500 x g) for 1 to 2 minutes. Discard the flow-through.
8. Repeat the wash step by adding a further 2.5ml solution WB. Centrifuge at high speed (minimum 3500 x g) for 1 to 2 minutes. Discard the flow-through.
9. Centrifuge at high speed (minimum 3500 x g) for 5 minutes to remove all traces of ethanol.
10. Place the column onto a clean 15ml collection tube. Add 400µl EB buffer pre-heated at 70^{0}C to the centre of the membrane.
11. Stand the column for 3 minutes then centrifuge at high speed (minimum 3500 x g) for 3 minutes to elute the DNA.
12. Check DNA concentration and purity by nanodrop or Qubit (Picogreen) assay.

Optional ethanol precipitation step for increasing DNA concentration

13. If the concentration is below the required level, perform an ethanol precipitation step on the eluted sample.
14. Place the 400µl eluted sample in a 2ml microcentrifuge tube. Add 40µl 3M Sodium Acetate pH5.2. Vortex briefly then add 1320µl (3 vols.) 98-100% ethanol, invert to mix. The DNA should be visible as white threads.
15. Centrifuge at maximum speed (13.4K rpm, 12,000g) for 3 minutes. Carefully pour off supernatant without disturbing the pellet. Wash with 1ml 70% ethanol. Invert several times to mix then centrifuge at maximum speed (13.4K rpm, 12,000g) for 1 minute. Carefully remove all of the liquid and dry briefly.
16. Re-hydrate the DNA pellet in 200µl TE buffer or EB buffer. Repeat the nanodrop scan or Qubit assay to check DNA concentration and purity.

DNA yields are typically well in excess of 20µg with DNA concentrations above 50ng/µl. Typical A260/280 ratios for the eluted DNA are >1.8, and A260/230 ratios are >1.5.