

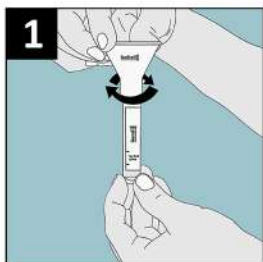
Patient Instructions for Saliva Collection

Do not eat, drink, smoke, brush your teeth or chew gum for 30 minutes before use.

Precautions: Do not swallow. If the stabilising solution comes into contact with the skin or eyes, wash with plenty of water. Tube cap may be a choking hazard to small children. MSDS available at www.isohelex.com

Kit Contents: Sample tube with 1ml stabilising solution, funnel and instruction sheet.

GeneFix™ RNA Saliva Collectors are intended for the collection of human saliva samples for RNA stabilisation.

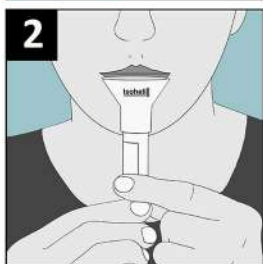
**Before use:**

Remove the collection funnel and tube from the pack.

Check that the level of the liquid in the tube is at the **1ml line** before use.

If the solution is cloudy, or crystals are present in the solution, warm the tube gently in your hands for a few minutes.

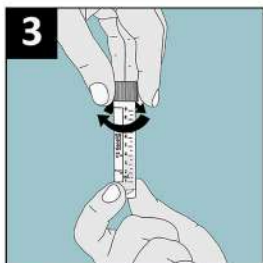
1. Unscrew the cap from the tube being careful not to spill the liquid. Keep the cap for use later. Screw the collection funnel on to the tube gently. **Do not over-tighten.**



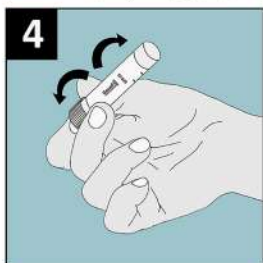
2. Start spitting into the funnel. **Tip: If the saliva does not flow easily into the tube, unscrew the funnel partially.**

Continue spitting into the funnel until the level of liquid in the tube, not including bubbles, reaches the **2ml Fill To** line.

To encourage saliva production you may rub the inside of your cheek with your tongue, or gently massage the outside of your cheeks.



3. Unscrew the collection funnel and replace the tube cap tightly. Discard the funnel.

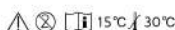


4. Shake the collection tube several times to mix the saliva with the solution. The sample can now be stored at room temperature for up to 14 days before RNA isolation.

For Laboratory Processing:

Proteinase K & RNA Precipitation reagent will be required for lysis. Please visit www.isohelex.com for more information.

For Research Use Only



Version September 2018

GeneFix Saliva RNA Collectors

Instructions for processing the stabilised saliva sample ready for RNA isolation

Reagents included with 50 x RFX-01 GeneFix Saliva RNA Collectors:

2 x Vial containing 22mg lyophilised Proteinase K: reconstitute each vial with 1.1ml RNase-free ddH₂O before use. Store at 4°C after reconstitution.

Bottle containing 5ml Precipitation Reagent: store at room temperature.

Equipment and reagents to be supplied by user:

RNase-free ddH₂O

RNase-free 1.5ml or 2ml microcentrifuge tubes

Waterbaths or heating blocks at 60°C and 90°C

98-100% Ethanol (ice cold)

Microcentrifuge with rotor for 1.5ml/2ml tubes

Vortexer

Before starting:

Reconstitute the vial of Proteinase K with 1.1ml RNase-free ddH₂O

Prepare waterbaths or heating blocks at 60°C and 90°C

Calculate the volumes of PK, Precipitation Reagent and ethanol to be added, depending on the volume of sample being isolated. The remainder of the sample can be stored for up to 14 days at room temperature, or frozen at -20°C or -80°C.

Instructions:

1. Vortex the saliva collection tube to mix. Remove 300µl - 500µl into a clean, RNase-free 1.5ml or 2ml tube.
2. Add 1/50th volume of Proteinase K @20mg/ml. Vortex to mix.
3. Incubate @60°C for 1 hour, then heat @ 90°C for 15 minutes and cool to room temperature.
4. Add 1/25 volume of Precipitation Reagent, vortex and incubate on ice for 10 minutes.
5. Spin @ 13.4K rpm/12,000 x g for 5 minutes.
6. Carefully remove the supernatant into a clean, RNase-free 1.5ml or 2ml tube.
7. Add 2 volumes of 98-100% ice cold Ethanol. Invert to mix then store @ -20°C for 30 minutes.
8. Spin @ 13.4K rpm/12,000 x g for 5 minutes.
9. Carefully remove all of the liquid without disturbing the pellet.
10. Dissolve the pellet in 300µl-500µl RNA lysis buffer from your RNA isolation kit of choice and proceed with RNA isolation as per the kit instructions.