Isohelix *Xtreme*-RNA Isolation Kit for Saliva/Swab Samples XMR-5/XMR-50: Instructions for Use

Product Details

The Isohelix *Xtreme*-RNA kit is a spin-column based RNA purification kit for the swift, simple preparation of total human, viral, or microbial RNA from saliva and swab samples. Free from toxic reagents such as phenol, chloroform, β-mercaptoethanol, & guanidine salts, Xtreme-RNA has been fully optimised for use with GeneFix GFX & RFX saliva collectors and swabs stored in BuccalFix stabilisation solution. Extracted samples are of high purity (expected A260/280: >1.9), ideal for use in downstream applications such as rt-qPCR and gene expression studies. The kit is also scalable and can accommodate various sample input volumes.

Kit Contents

Catalogue Number:	**XMR-5**	**XMR-50**	**Storage Temperature**
Number of samples processed	5 x 500µl aliquots	50 x 500µl aliquots	
Contents:			
Proteinase K [20 mg/ml]	2.2mg[1]	11mg[2]	4°C after reconstitution
Precipitation Solution (PE)	1.25 ml	12.5 ml	Room Temperature
RNA Spin-Columns	5 columns	50 columns	Room Temperature
Wash Solution (WS)	1.9ml [3]	19 ml[4]	Room Temperature
Elution Solution (ES)	500 µl	5 ml	Room Temperature
Protocol			

(1) Reconstitute vial with 110µl RNAse free H_2O before first use, store at 4°C after reconstitution.
(2) Reconstitute vial with 550µl RNAse free H_2O before first use, store at 4°C after reconstitution.
(3) Add 4.5ml >98% Molecular Biology grade Ethanol into solution WB before first use, tighten the cap securely to prevent evaporation.
(4) Add 45ml >98% Molecular Biology grade Ethanol into solution WB before first use, tighten the cap securely to prevent evaporation.

Storage

Isohelix *Xtreme*-RNA 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 & Reagents to be supplied by user

- Dry heating blocks set at 60°C & 90°C.
- Laboratory cool/chill block to fit 1.5ml/2.0ml tubes. A -20°C freezer can be used as an alternative.
- Pipettes (10µl, 200µl, & 1000µl) with disposable RNAse-free filter tips.
- Disposable nitrile gloves.
- Microcentrifuge capable of RCF = 12,000 x g (with rotor for 1.5/2.0 ml tubes).
- 1.5ml & 2.0ml RNAse-free microcentrifuge tubes.
- Vortexer
- Molecular Biology grade >96% Ethanol & >99% Isopropanol (2-Propanol).
- RNAse-free H_2O

Important Notes on RNA Sample Handling:

RNA species by nature are inherently fragile and liable to physical degradation and from digestion by ubiquitous RNAse enzymes present in the environment. It is therefore important that care is taken to avoid this in order to maximise RNA sample quality. The following notes are recommendations to help prevent this:

I. Set aside a separate area for RNA isolation away from other work, using separate sets of pipettes, pipette tips, gloves, and other consumables. Ideally a UV decontamination cabinet or laminar flow hood should be used.
II. Always wear a clean lab coat, gloves, and a face mask while handling samples. These will help prevent introduction of RNAses from the user to the workspace.
III. Prior to starting, wipe down all work surfaces and clean pipettes with a 10% household bleach solution (or any other commercially available RNAse decontamination solution), followed by wiping down with RNAse-free/DEPC-treated H_2O to inactivate potential RNAses. Common disinfectants such as 70% Isopropanol may be insufficient for inactivating RNAses.
IV. Always use consumables and reagents that are molecular biology grade or certified as RNAse/Nuclease free. Lower-quality reagents may be contaminated with RNAses.
V. Once begun, the protocol should not be paused, and the user should work as swiftly as reasonably possible to complete sample purification. Purified samples should always be kept chilled or on ice.

Before Starting

1. Preheat heating blocks to 60°C & 90°C, respectively.
2. Reconstitute the Proteinase K by adding the appropriate volume of H_2O defined on the kit contents table.
3. Add the appropriate volume of >98% Ethanol to the Wash Solution (WB) bottle as defined on the kit contents table.
4. Vortex collection tubes containing samples briefly prior to use.

Safety & Use of the *Xtreme*-RNA Isolation Kit

The Isohelix *Xtreme*-RNA Isolation kits are intended for use by qualified professionals trained in potential laboratory hazards and good laboratory practice. Lab coats, gloves, and eye protection should be worn while using this kit. This kit has been designed for research use only.

Protocol for RNA Isolation from 250µl aliquot Stabilised Saliva or Swab sample:

1. Aliquot 250µl of sample into a fresh 2.0ml centrifuge tube. Add 5µl of Proteinase K solution and vortex briefly to mix. Incubate sample(s) at 60°C for 30-60 minutes, followed by 15 minutes at 90°C. Following this, immediately chill samples for 1 minute to cool, then place at room temperature.

2. Add 125µl of Precipitation Solution (PE) to the sample(s), vortexing to mix. At this point the samples will appear cloudy. Place sample(s) in a microcentrifuge and spin at 12,000 x g for 10 minutes.

3. Carefully recover 350µl of supernatant from the sample tube(s) and dispense into a fresh 2.0ml centrifuge tube, taking care not to disturb the pellet containing impurities.
Note: If the pellet is disturbed briefly re-spin the tubes to resettle the sample(s).

4. Pipette 350µl of >99% Isopropanol into the sample tube(s) and vortex to mix. Prepare RNA spin column(s) and load the solution from each sample (700µl) onto it. Centrifuge the column(s) for 1 minute at 12,000 x g. Discard the flow-through.

5. Add 600µl Wash Solution (WS) onto each column and spin for 1 minute. Discard the flow-through.

6. Add a further 600µl Wash Solution onto each column, then spin again for 3 minutes, discarding the flow-through. If residual wash solution is present in or around the tube, repeat the spin for 1 minute to remove.

7. Place the column(s) in clean 1.5ml centrifuge tube(s). Add 50µl of Elution Solution (ES) to the centre of the column membrane. Stand the column(s) for 1 minute, then centrifuge for 1 minute to elute the RNA.
Note: The volume of elution solution added can be increased up to 100µl if a higher total yield is needed or decreased down to 30µl if a higher sample concentration is required.

8. Immediately chill the sample on a chill block or ice. The RNA sample(s) are now purified and can be quantified for purity & yield by Nanodrop/UV photometer and Qubit fluorometric assay. The eluted RNA can be stored short term at -20°C for up to a week, or for longer term at -80°C. For best results use samples as soon as possible after isolation.

Protocol for RNA Isolation from 500µl aliquot Stabilised Saliva or Swab sample:

1. Aliquot 500µl of sample into a fresh 2.0ml centrifuge tube. Add 10µl of Proteinase K solution and vortex briefly to mix. Incubate sample(s) at 60°C for 30-60 minutes, followed by 15 minutes at 90°C. Following this, immediately chill samples for 1 minute to cool, then place at room temperature.

2. Add 250µl of Precipitation Solution (PE) to the sample(s), vortexing to mix. At this point the samples will appear cloudy. Place sample(s) in a microcentrifuge and spin at 12,000 x g for 10 minutes.

3. Carefully recover 700µl of supernatant from the sample tube(s) and dispense into a fresh 2.0ml centrifuge tube, taking care not to disturb the pellet containing impurities.
Note: If the pellet is disturbed briefly re-spin the tubes to resettle the sample(s).

4. Pipette 700µl of >99% Isopropanol into the sample tube(s) and vortex to mix. Prepare RNA spin column(s) and load the solution from each sample (700µl) onto it. Centrifuge the column(s) for 1 minute at 12,000 x g. Discard the flow-through. Repeat this step as necessary until the entire sample has been loaded.

5. Add 600µl Wash Solution (WS) onto each column and spin for 1 minute. Discard the flow-through.

6. Add a further 600µl Wash Solution onto each column, then spin again for 3 minutes, discarding the flow-through. If residual wash solution is present in or around the tube, repeat the spin for 1 minute to remove.

7. Place the column(s) in clean 1.5ml centrifuge tube(s). Add 100µl of Elution Solution (ES) to the centre of the column membrane. Stand the column(s) for 1 minute, then centrifuge for 1 minute to elute the RNA.
Note: The volume of elution solution added can be decreased down to 30µl if a higher sample concentration is required.

8. Immediately chill the sample on a chill block or ice. The RNA sample(s) are now purified and can be quantified for purity & yield by Nanodrop/UV photometer and Qubit fluorometric assay. The eluted RNA can be stored short term at -20°C for up to a week, or for longer term at -80°C. For best results use samples as soon as possible after isolation.

DNA Removal Note:

Residual DNA will co-elute with RNA extracted when using this kit. Some sensitive downstream RNA applications will require a DNAse treatment step to remove any remaining DNA. If required, DNAse digestion can be performed following final elution of RNA using most commercially available DNAse kits.