**Canvax Reagents, S.L.U.**
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# DATA SHEET

Version: 02
Revision date: 26/05/2023

## 1. Identification

**Product name** **HigherPurity™ Saliva RNA Extraction Kit**

**Cat. No** **AN0077 (50 reactions)**
**Cat. No** **AN0077-XL (250 reactions)**

## 2. Description

**HigherPurity™ Saliva RNA Extraction Kit** provides an efficient method for RNA extraction from preserved saliva samples in our **RNA-Saliva sample collection & stabilization kit** (recommended-not included). The procedure includes cell lysis, protein removing, RNA precipitation, washing and hydration. Finally, for removal of genomic DNA contamination is used an approach consisting of two sequential filtrations with different MiroSpin columns.

## 3. Composition

| Item | Quantity | |
|---|---|---|
| | AN0077 | AN0077-XL |
| Precipitation Solution | 12 ml | 60 ml |
| Binding Buffer | 22 ml | 110 ml |
| WB1 Buffer* | 10 ml | 50 ml |
| RNA miniSpin Columns | 50 Units | 250 Units |
| Collection tubes | 100 Units | 500 Units |
| gDNA Removal columns | 50 Units | 250 Units |
| Proteinase K** | 30 mg | 5 x 30 mg |
| Nuclease-Free water | 8 ml | 40 ml |

*Add the volume ethanol (96%-100%) specified [Not included] to WB1 buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

**Dissolve Proteinase K in 1.3 ml of Nuclease-Free water to obtain a stock solution.

## 4. Storage

Store the kit at room temperature. If any kit reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves and allow to cool to room temperature before use.

**The Proteinase K solution can be stored for several days at 4°C**. For longer-term storage, the unused portion of the solution may be stored in aliquots at – 20 °C until needed.

## 5. Features

- No phenol-chloroform extraction.
- Buffer-based RNA isolation combined with gDNA removal with columns.
- Sample Volume: 600 ul of preserved saliva sample.
- Reduces sample collection distress and blood sample handling.

## 6. Applications

- Purification of total RNA from saliva of different origins (human or animal).

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## 7. Quality Certifications

**HigherPurity™ Saliva RNA Extraction Kit** is analysed by a Total RNA extraction. RNA purified is analysed by electrophoresis.

## 8. Quality Certifications

**HigherPurity™ Blood DNA Extraction kit** is tested on a lotto-lot basis by isolating total DNA from 200µl of whole human blood. DNA purified is analyzed by:

- Spectrophotometer: Ratio 260/ 280 (1.6-1.8)
- Agarose gel electrophoresis.

## 9. Further information

| | |
|---|---|
| **Product Use Limitations** | This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. |
| **Safety Information** | When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit. |

## DETAILED PROTOCOL

### Tota RNA isolation

**1.** Mix well the tube containing 1 ml of preserved saliva sample in the RNA-Saliva sample collection & stabilization kit (recommended-not included). *It is important to see a homogeneous solution.*
**2.** In a microtube with 25 µl of **Proteinase K** add 600 µl of preserved saliva. Incubate at 55˚C for 45 minutes.
**3.** Incubate at 90˚C for 15 minutes.
**4.** Place the microtubes at 4˚C for 10 minutes.
**5.** Add 200 µl **Precipitation Solution**. Vortex and centrifuge at 14.000 rpm for 5 minutes.
**6.** Transfer the supernatant in a new 1.5 ml microtube containing 600 µl of Isopropanol (not provided).
**7.** Centrifuge at 14.000 rpm for 3 minutes and remove the supernatant. *The DNA will be visible as a small white pellet.*
**8.** Wash the RNA pellet with 600 µL 70%ethanol (not provided) to remove residual isopropanol and centrifuge at 14.000 rpm for 1 minute. Carefully remove the supernatant without touching the RNA pellet. It can be re-centrifuged briefly to collect the last drops.

### Removal gDNA contaminant

**1.** Add 400 µl of **Binding Buffer** and resuspend well the RNA pellet with micropipette.
**2.** Incubate at 55˚C for 15 minutes.
**3.** Transfer the sample to a **gDNA removal column** and place the column into a collection tube.
**4.** Centrifuge for 1 minute at 10.000 rpm.
**5.** Add 400 µl of 100% ethanol to the supernatant collected in the previous step (4). Mix well.
**6.** Place an **RNA-miniSpin Column** in a Collection tube and transfer the mixture to the column.
**7.** Centrifuge at 8.000-10.000 rpm for 1 minute. Pass the sample twice as the volume exceeds the capacity of the column.
**8.** Add 700 µl **WB1 Buffer**. Centrifuge at maximum speed for 1 minute.
**9.** Again, Centrifuge at full speed for 3 minutes. This step helps to dry the RNA miniSpin column.
**10.** Place the RNA miniSpin column into a new, labelled 1.5 microcentrifuge tube and pipet 50µl of **Nuclease-Free water** directly into the centre of the membrane. Close the cap and incubate for 2 minutes at room temperature. Centrifuge at full speed for 1 minute to elute RNA.