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

Version: 03
Revision date: 27/03/2023

## 1. Identification

**Product name** **HigherPurity™ Tissue Total RNA Purification Kit**

**Cat. No.** **AN0150 (50 reactions)**
**Cat. No.** **AN0152 (100 reactions)**
**Cat. No.** **AN0152-XL (250 reactions)**

## 2. Description

**HigherPurity™ Tissue Total RNA Purification Kit** offers a rapid and convenient method for purification of total RNA from a variety of tissue and culture cells. The kit is based in nucleic acid ability to bind silica in the presence of high concentrations of chaotropic salts. Tissue samples can be efficiently homogenized in a microcentrifuge tube using the provided micropestle. Eluted purified RNA is ready for use in a variety of downstream applications: real-time RT-PCR, Northern Blotting, cDNA library construction, etc.

## 3. Kit Contents

| Items | Quantity | | |
|---|---|---|---|
| | AN0150 (50 rxn) | AN0152 (100 rxn) | AN0152-XL (250 rxn) |
| Buffer BLY * | 20 ml | 40 ml | 100 ml |
| Wash Buffer 1 | 27 ml | 54 ml | 135 ml |
| Wash Buffer 2 ** (WB2) | 16 ml | 32 ml | 80 ml |
| RNase-free ddH2O | 5 ml | 8 ml | 15 ml |
| RNAprep Spin Columns | 50 | 100 | 250 |
| Filter Column | 50 | 100 | 250 |
| Collection tube (2mL) | 100 | 200 | 500 |
| 1.5 ml microcentrifuge tube | 50 | 100 | 250 |
| Micropestle | 50 | 100 | 250 |

* Before beginning the lysis and homogenization steps, prepare a fresh amount of Buffer BLY containing 1% 2-mercaptoethanol (β-ME) [Not included] for each purification procedure. Add 10 µL β-ME for each 1 mL Lysis Buffer (Buffer BLY).

**Add the volume ethanol (96%-100%) specified [Not included] to WB2 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.

**! Caution:** Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.

## 4. Storage specifications

All components can be stored at Room Temperature.

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## 5. Applications

Purified RNA suitable for all molecular biology applications, such as:

- RT-PCR.
- Northern blotting.
- cDNA library construction.

## 6. Quality Certifications

Total RNA is isolated from a 30 mg thorax muscle tissue sample. Purified RNA is quantified using a spectrophotometer with a typical yield of more than 10µg of total RNA and a A260nm/A280nm ratio of 1.9-2.1. Quality is further checked by agarose gel electrophoresis.

## 7. 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. |

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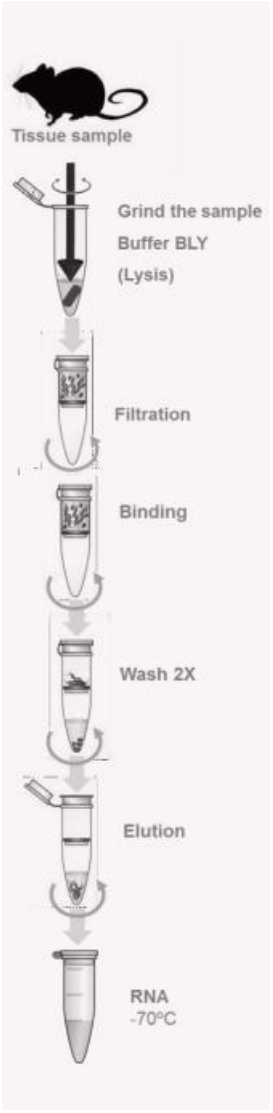
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## 8. Assay Procedure




1. Cut up to 30mg of animal tissue and transfer to a 1.5-ml microcentrifuge tube (not provided). Use the **micropestle** to grind the material to pulp (or a motor-driven grinder). You can grind the tissue sample in liquid nitrogen.
2. Add **350µl of Buffer BLY** (β-ME added) and continue to homogenize the sample by grinding. In order to release all RNA in the sample, it is required to disrupt the sample completely.
3. Incubate at room temperature for 5 minutes.
4. Place a **Filter Column** in a 2 ml **Collection tube** and transfer the sample mixture to the filter column. Centrifuge at 10,000 g for 2 minutes.
5. Carefully transfer the clarified filtrate to a **new 1.5 ml microcentrifuge tube**.
6. Add 1 volume of 70% ethanol to the clarified lysate and mix vigorously by vortexing.
7. Apply the total volume (usually 700 µl) from step 6 to the **RNAprep spin column** by decanting or pipetting.
8. Centrifuge at 10,000 g for 90 seconds. Discard the flow-through.
9. **[Optional]** To eliminate genomic DNA contamination, follow the steps from a. to d. Otherwise, proceed to step 10 directly:
   a. Add **250 µl of WB1** to wash RNAprep spin column. Centrifuge at full speed for 1 min then discard the flow-through.
   b. Add 60 µl of RNase-free DNase 1 solution (0.5U/µl, not provided) to the membrane centre of RNAprep spin column. Let stand for 15 minutes at room temperature. (***)
   c. Add **250 µl of WB1** 1 to wash RNAprep spin column. Centrifuge at full speed for 1 min then discard the flow-through.
   d. After DNase 1 treatment, proceed to step 11.

   *** *We recommend the use of On-membrane DNase I Set: Cat. No: EZ0020*
10. Add **500 µl of WB1** and centrifuge at full speed for 30 seconds. Discard the flowthrough.
11. Add **750 µl of WB2** and centrifuge at full speed for 30 seconds. Discard the flow-through.
12. Repeat this step 11.
13. Again, Centrifuge at full speed for 2 minutes. This step helps to dry the RNAprep spin column.
14. Place the RNAprep spin column into a new, labelled 1.5 microcentrifuge tube and pipet 40-50µl of **RNase-free ddH2O** directly into the center of the column membrane. Close the cap and incubate for 1 minute at room temperature.
15. Centrifuge at full speed for 1 minute to elute RNA.
16. Keep eluted RNA on ice at all times and store at <-70˚C.