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# One Prep DNA/RNA Purification Kit

**(Cat# K2058-50; 50 preparations, store at RT)**

## I. Introduction:

**BioVision's One Prep DNA/RNA Purification Kit** allows the quick and simple extraction of genomic DNA and RNA from the same mammalian sample including cultured cells or tissues. The purified DNA and RNA are suitable for numerous downstream applications including RT-PCR, next generation sequencing etc.

## II. Application:

- Purification of DNA and RNA from mammalian cells and tissues.

## III. Key Features:

- DNA and RNA purification from the same sample.
- Purified DNA and RNA are suitable for numerous downstream applications.

## IV. Sample Type:

- Up to $1x10^7$ (cultured cells) or 20 mg (tissue)

## V. Kit Contents:

| Components | 50 preparations | Cap Color | Part Number |
|---|---|---|---|
| RNA-Lysis Buffer | 45 ml | Amber/WM | K2058-50-1 |
| *RW1 Buffer | 40 ml | NM | K2058-50-2 |
| *W2 Buffer | 22.5 ml | NM | K2058-50-3 |
| RNase-free water | 10 ml | NM | K2058-50-4 |
| *DW1 Buffer | 11 ml | WM | K2058-50-5 |
| DNA Elution Buffer | 10 ml | WM | K2058-50-6 |
| RNA Columns | 50 | -- | K2058-50-7 |
| DNA Columns | 50 | -- | K2058-50-8 |

**Add Ethanol to RW1, W2 and DW1 Buffer bottles before use.*

## VI. User Supplied Reagents and Equipment:

- 96-100% Ethanol; 70% Ethanol
- β-mercaptoethanol (14.3 M)
- Barrier pipette tips
- 1.5 ml and 2 ml nuclease-free microcentrifuge tubes
- Benchtop centrifuge
- Syringe with 25 gauge needle and/or Dounce Homogenizer (BioVision Cat# 1998)

## VII. Storage Conditions and Reagent Preparation:

Store all the reagents at room temperature (RT) for up to a minimum of 1 year, protected from light.

- ***RW1 Buffer:** Add 10 ml of (96-100%) Ethanol to RW1 Buffer bottle before first use and mix well.
- ***W2 Buffer:** Add 90 ml of (96-100%) Ethanol to W2 Buffer bottle before first use and mix well.
- ***DW1 Buffer:** Add 15 ml of (96-100%) Ethanol to DW1 Buffer bottle before first use and mix well.
- **RNA-Lysis Buffer:** Determine the amount of RNA-Lysis Buffer required and add 10 µl of β-mercaptoethanol (β-ME) per 1 ml RNA-Lysis Buffer for immediate use. Prepare fresh. **Note:** β-ME is recommended for RNase rich cell lines. We recommend using 350 µl RNA-Lysis Buffer for 1-5 x $10^6$ cells and 600 µl RNA-Lysis Buffer for 5-10 x $10^6$ cells respectively.

## VIII. Purification Protocol:

**1. Sample Lysis:**

- **Suspension cells:** Collect cells by centrifuging at 300 x *g* and RT for 5 min. Remove the supernatant completely by aspiration and add RNA-Lysis Buffer.
- **Adherent cells:** Aspirate the media completely with a Pasteur pipet and add RNA-Lysis Buffer to the flask. Scrape the cells and move the suspension to a microcentrifuge tube.

**Notes:** i) Growth media must be removed completely. Residual media will inhibit cell lysis and affect the RNA yield. ii) Adherent cells can alternatively be treated with enzymes, pelleted and treated like suspension cells.

1) Lyse cells by pipetting up and down and passing the sample through a 25 gauge needle at least 5-10 times.

2) Ensure minimal sample loss and adjust the volume using RNA-Lysis Buffer, if needed. Entire lysate can be used for Total RNA Purification.

- **Tissue samples:**

Remove the preservation solution completely and weigh the tissue sample. Add 600 µl RNA-Lysis Buffer. **Note:** Sample should not exceed 20 mg.

1) Homogenize sample(s) by using a Dounce Homogenizer or mortar and pestle or by any other method of choice.

2) Lyse sample further by pipetting up and down and passing the sample through a 25 g needle at least 5-10 times.

2) Centrifuge at 13,000 x *g* and RT for 5 min to remove any insoluble material. Clarified lysate (supernatant) is used for Total RNA Purification.

**Notes:** i) Ensure minimal sample loss and adjust the volume using RNA-Lysis Buffer, if needed. ii) Fatty tissue lysates should be centrifuged for an additional 5 min at 13,000 x *g*.

**2. Total RNA Purification:**

1) Load the lysate on a DNA Column and centrifuge at 10,000 x *g* and RT for 30 sec and collect the flow through.

2) Move the DNA Column to a fresh 2 ml nuclease-free microcentrifuge tube and save for **DNA Purification**.

3) Add 1 volume of 70% Ethanol to the flow through and mix by pipetting. A precipitate may form but will not interfere with purification.

4) Transfer 700 µl of the solution to a RNA column and centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow-through. **Note:** If any solution remains, repeat step 4.

5) Add 700 µl of RW1 Buffer ***(*ensure ethanol was added)*** to the column and centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow through***. Optional: On-Column DNA Digestion protocol using (BioVision Cat# K2066) may be performed instead of step 5.***
6) Add 500 µl W2 Buffer ***(*ensure that ethanol was added)*** to the column and centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow through.
7) Add another 500 µl of W2 Buffer to the column and centrifuge at 10,000 x *g* and RT for 1 min.
8) Place the column in a 2 ml nuclease-free microcentrifuge tube. Centrifuge the column at maximum speed and RT for 1 min.
9) Immediately transfer the column to a new 1.5 ml nuclease-free microcentrifuge tube. Pipette 50 µl of RNase-free water to the center of the column. Centrifuge at 10,000 x *g* and RT for 1 min to elute the RNA.
10) Store the RNA solution at -70 °C or -20 °C for short term storage.
**Note:** For quantification by spectroscopy, an aliquot of RNA should be diluted with 10 mM Tris-HCl, pH 7.5.

**3. Genomic DNA Purification:**

1) Add 500 µl DW1 Buffer ***(*ensure that ethanol was added)*** to the saved DNA column. Centrifuge at 10,000 x *g* and RT for 30 sec and discard the flow through.
2) Add 500 µl W2 Buffer to the column and centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow through.
3) Add another 500 µl W2 Buffer to the column and centrifuge at 10,000 x *g* and RT for 1 min.
4) Place the column into a new 2 ml centrifuge tube and centrifuge at a maximum speed for 1 min.
5) Transfer the column to a new 1.5 ml microcentrifuge tube. Pipette 100 µl of DNA Elution Buffer to the center of the column. Incubate for 1 min and centrifuge at 10,000 x *g* and RT for 1 min to elute the DNA.
6) Store DNA solution at -20 °C.

***I.*** General Troubleshooting Guide:

| Low $A_{260}/A_{280}$ ratio | • Incorrect blank<br>• Protein contamination | • Ensure that RNA samples are diluted in 10 mM Tris, pH 7.5, and dilution solution is used for blank.<br>• Perform Phenol:Chloroform extraction. Loss of total nucleic acids (up to 40%) should be expected. Add 2.5 volumes of ethanol and 0.1 M NaCl to precipitate the nucleic acids. Incubate for 30 min at -20 °C. Centrifuge at 10,000 g for 15 min at 4 °C. Resuspend the RNA pellet in RNase-free water, DNA in Elution Buffer or TE Buffer, pH 8.5. |
|---|---|---|
| Low Yield | • Not enough sample<br>• Insufficient homogenization.<br>• Degraded sample(s).<br>• The binding capacity of the spin column was exceeded<br>• Ethanol was not added to wash buffer<br>• Ethanol carryover | • Increase the sample size.<br>• Ensure that the samples are homogenized well.<br>• Perform DNA and RNA purification using fresh samples.<br>• Split sample over multiple columns to avoid exceeding the column capacity.<br>• Make sure to add ethanol to the buffers before use (as instructed).<br>• Make sure that the column is dry before the elution step. |
| Salt contamination | • Salt carryover | • Dab top of collection tube with Kim wipe or paper towel after emptying<br>• Perform all suggested wash steps.<br>• Make sure the column is dry before the elution step. |
| RNA contamination of genomic DNA | • Ethanol | • Ethanol in the cell lysate will cause RNA to bind to DNA column. Make sure that ethanol is only added after passing the solution through the DNA column.<br>• Treat sample with DNase-free RNase and perform the clean-up step. |
| Genomic DNA contamination of RNA | • Sample too large.<br>• DNA column overloaded | • Reduce total number of cells used in lysis.<br>• Reduce the amount of starting tissue in the preparation of the homogenate. Most tissues will not show a genomic DNA contamination problem at 20 mg or less per prep.<br>• Perform an on the column DNA digestion or DNA digestion followed by an RNA clean-Up |

**IX. Functional Test Data:**

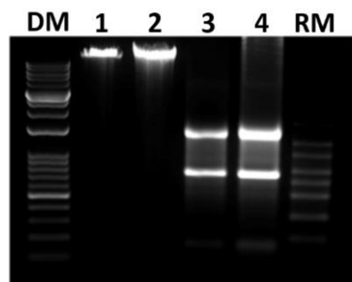


**Figure 1: DNA and RNA were purified using One Prep DNA/RNA Purification Kit.** Genomic DNA (lanes 1 & 2) and RNA (lanes 3 & 4) were purified from the same 5 x $10^6$ and 1x$10^7$ Jurkat cells stored in RNAkeep™ Solution (BV Cat# M1241) respectively. 10 µl of each sample was analyzed on a 1% agarose gel. DM: 1 kb DNA Ladder (BV Cat# M1157); RM: Riboruler.

**X. Related Products:**

Mammalian RNA Isolation Kit (K2065)
RNAkeep™ Solution (M1241)
EasyRNA™ Blood RNA Mini Kit (K1373)
Yeast RNA Mini Kit (K1418)
On-Column DNase Digestion Kit (K2066)
EasyRNA™ Bacterial RNA Kit (K1351)
EasyRNA™ Plant RNA Mini Kit (K1374)
EasyRNA™ Blood RNA Mini Kit (K1373)

***FOR RESEARCH USE ONLY! Not to be used on humans.***