On-Column DNase Digestion Kit

(Cat# K2066-50, 50 preparations, Store at -20 °C)

I. Introduction:

BioVision's On-Column DNase Digestion Kit provides the user a quick and efficient way to remove contaminating genomic DNA from RNA preparations. The kit is designed to be used in conjunction with BioVision's Mammalian RNA Isolation Kit (Cat# K2065) or One Prep DNA/RNA Purification Kit (Cat# K2058) to clean-up or eliminate DNA from purified RNA or from enzymatic reactions. The purified RNA is suitable for numerous downstream applications including RT-PCR. Additionally, the digestion protocol can be used with other commercially available RNA extraction kits.

II. Application:

- Removal of trace amounts of DNA from RNA being purified using spin-column technology.

III. Key Features:

- On-column digestion of DNA
- DNA digestion prior to RNA clean-up

IV. Sample Types:

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

V. Kit Contents:

Components	50 preparations	Cap Code	Part Number
RNase-free DNase	100 µl	Red	K2066-50-1
10X RNase-free DNase Buffer	500 µl	Yellow	K2066-50-2
RNase-free Water	5 ml	NM	K2066-50-3

VI. User Supplied Reagents and Equipment:

- Ethanol (96-100%)
- Barrier pipette tips
- 1.5 ml RNase free microcentrifuge tubes
- Benchtop centrifuge
- One Prep DNA/RNA Purification Kit **(BioVision Cat# K2058)**
- Mammalian RNA Isolation Kit **(BioVision Cat# K2065)**

VII. Shipment and Storage:

The kit is shipped at 4 °C but should be stored at -20 °C. Aliquots of RNase-free DNase should be stored at -20 °C but can be stored at 4 °C for up to 1 month. Avoid repeated freeze-thaw cycles.

VIII. DNA Digestion Protocols:

On-Column DNA Digestion *(after step 4 of Total RNA Purification Protocol under section VIII of BV Cat# K2058 or # K2065))*

1. Prepare the **DNA digestion master mix** containing 5 µl of 10X RNase-free DNase Buffer, 43 µl of RNase-free water and 2 µl of RNase-free DNase. Mix well. **Note:** When performing multiple digestions, mix enough reagents for the number of digestions.
2. Add 350 µl of RW1 Buffer to the RNA Column and centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow through.
3. Load 50 µl of DNA digestion master mix in the center of the RNA Column. Incubate at room temperature (RT) for 15 min to remove any trace amounts of genomic DNA.
4. Add 350 µl RW1 Buffer to the RNA Column and centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow-through
5. Return back to steps 6-10 of the **Total RNA Purification Protocol** (BV # K2058 or BV # K2065).

RNA Clean-Up Protocol *(use either with BV # K2058 or BV # K2065)*

1. Add RNase-free water to the enzymatic reaction to adjust the final volume to 100 µl.
2. Add 350 µl of RNA-Lysis buffer to the diluted RNA and mix well.
3. Add 250 µl of Ethanol (96-100%) and mix well.
4. Load the 700 µl of the solution onto an RNA column and centrifuge at 10,000 *x g* and RT for 30 sec. Discard the flow-through. **Perform Optional On-Column DNA digestion, if required.**
5. Add 500 µl RW2 (or W2) Buffer (*add Ethanol to RW2 or W2 Buffer before use) to the column and centrifuge at 10,000 *x g* and RT for 30 sec. Discard the flow-through.
6. Add another 500 µl RW2 (or W2) Buffer to the column and centrifuge at 10,000 x g at RT for 1 min.
7. Put the column in a new collection tube. Centrifuge the column at max speed for 1 min.
8. Transfer the column to an RNase-free 1.5 ml tube and add 50-100 µL RNase free water to the center of the column. Centrifuge at 10,000 x *g* and RT for 1 min to elute the RNA.
9. Store the RNA solution at -80 °C or -20 °C for short term storage. To quantify using spectroscopy, RNA should be diluted in 10 mM Tris-HCl, pH 7.5.

Alternate RNA Clean-Up and DNA Digestion Protocol

1. Add 10 µl 10X RNase-free DNase Buffer and 2 µl RNase-free DNase to the RNA enzymatic reaction. Adjust the volume to 100 µl with RNase-free water and mix well.
2. Incubate at 37 °C for 15-30 min.
3. Add 350 µl of RNA-Lysis Buffer and mix well.
4. Add 250 µl of Ethanol (96-100%) and mix well.
5. Load 700 µl of the solution onto an RNA Column and centrifuge at 10,000 x *g* and RT for 30 sec. Discard the flow-through.
6. Return to steps 6-10 the **Total RNA Purification Protocol** (BV # K2065 or BV # K2058) or steps 5-9 of the **RNA Clean-Up Protocol** above.

I. General Troubleshooting Guide:

Low A_{260}/A_{280} ratio	• Protein contamination	• Perform all the wash steps, particularly with RW1 buffer.
	• Low yield	• Ensure that the sample is diluted in 10 mM Tris, pH 7.5, and the dilution solution is used as blank.
Low Yield	• Degraded sample	• Purification should be performed using fresh samples or well-preserved samples.
	• Poor column binding	• Make sure Ethanol is added and the sample is mixed well before binding to the column.
	• Exceeding the column capacity	• It is best to split the samples when the RNA yield is > 50 µg.
	• RNase contamination	• Be careful in preventing RNase contamination.

IX. Functional Test Data:

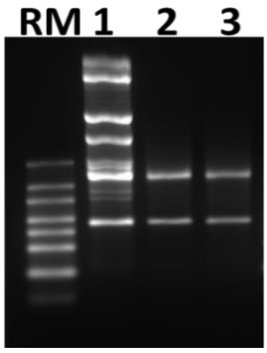

Figure 1: 5 µg Jurkat cell RNA mixed with Hind III-digested λ DNA was purified. 1. RNA without DNase treatment. **2.** DNase treatment prior to the RNA Clean-Up Protocol. **3.** RNA Clean-Up Protocol with optional On-Column DNA digestion step. **RM:** Riboruler marker. All samples were incubated at RT for 30 min for DNase treatment. 10 µl was loaded per lane.

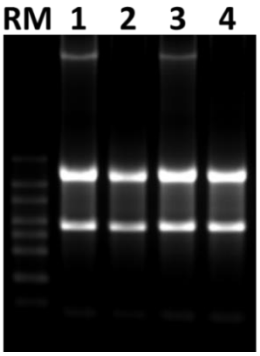

Figure 2: RNA was purified from Jurkat cells using BV Cat# K2065. Lanes 1 & 3. RNA without DNase treatment. **Lanes 2 & 4.** RNA with DNase treatment. **RM:** Riboruler marker. All samples were incubated at RT for 15 min for DNase treatment. 10 µl of elution was loaded per lane.

X. Related Products:

One Prep DNA/RNA Purification Kit (Cat# K2058)
Mammalian RNA Isolation Kit (Cat# K2065)
RNAkeep™ Solution (Cat# M1241)
EasyRNA™ Bacterial RNA Kit (Cat# K1351)
EasyRNA™ Blood RNA Mini Kit (Cat# K1373)
EasyRNA™ Plant RNA Mini Kit (Cat# K1374)
Yeast RNA Mini Kit (Cat# K1418)
EasyRNA™ Fungal RNA Kit (Cat# K1419)

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