User Information - DNA Removal Add-on

Residual DNA should be removed from FFPE RNA prior to rRNA depletion. Most FFPE RNA extraction kits contain a DNase I treatment, however, this may not be complete, depending on the amount of DNA that was co-purified. Lexogen offers a DNA Removal Add-on that can be seamlessly integrated upstream of RiboCop HMR V2 rRNA depletion. There is no need to purify samples between DNA removal and rRNA depletion. Total FFPE RNA from 5 ng - 1 µg can be used as input for DNA removal.

ATTENTION: Before starting this protocol, please read the General Guidelines for Lexogen Kits, which are available online. These provide a detailed overview of RNA and kit component handling, as well as general RNA input requirements.

1. Kit Components

DNA Removal Add-on

Kit Component	Tube Label	Volume*			Storage
		24 preps	96 preps	384 preps	
10x DNA Removal Buffer	**DRB** ●	27 µl	106 µl	4x 106 µl	-20 °C
DNA Removal Enzyme	**DRE** ●	27 µl	106 µl	4x 106 µl	-20 °C

*including ≥10 % surplus

2. User-Supplied Consumables and Equipment

Reagents

- RNase-free water.

Equipment

- Benchtop centrifuge (12,000 x g, rotor compatible with 1.5 ml tubes or 3,000 x g, rotor compatible with 96-well plates).
- Calibrated single-channel and multi-channel pipettes for handling 1 µl to 1,000 µl volumes.
- Thermocycler.
- UV-spectrophotometer to quantify RNA.
- Ice bath, ice box, ice pellets, or benchtop cooler (-20 °C for enzymes).

Labware

- Suitable low-binding pipette tips (pipette tips with aerosol barriers recommended).
- 1.5 ml reaction tubes, low binding, certified ribonuclease-free.
- 200 µl PCR tubes or 96-well plates and caps or sealing foil.
- Vortex mixer.

Optional Equipment

- Automated microfluidic electrophoresis station (e.g., Agilent Technologies 2100 Bioanalyzer).
- Benchtop fluorometer and appropriate assays (for RNA quality control).
- Agarose gels, dyes, and electrophoresis rig (for RNA quality control).

3. Short Protocol

DNA Removal
DRB ● – thawed at RT **DRE** ● – keep on ice or at -20 °C
Thermocycler 37 °C, 10 min

Up to 8 µl total FFPE RNA, ranging from 5 ng - 1 µg can be inserted into the DNA removal step.
ATTENTION: For the preparation of mastermixes include a 10 % surplus per reaction.

EXAMPLE: Step 1 for 24 preps: 26.4 µl **DRB** ● (= 1 µl x 24 rxn x 1.1)
+ 26.4 µl **DRE** ● (= 1 µl x 24 rxn x 1.1)
resulting in a total of 52.8 µl, , which is sufficient for multi-channel pipetting.

1	Prepare a mastermix of 1 µl 10x DNA Removal Buffer (**DRB** ●) and 1 µl DNA Removal Enzyme (**DRE** ●) per sample. Mix thoroughly and spin down briefly.
2	Add 2 µl of the **DRB / DRE** mastermix to 8 µl of FFPE RNA sample. If a smaller volume of RNA is used, add RNase-free water to a total reaction volume of 10 µl. Mix thoroughly and quickly spin down.
3	Incubate for 10 minutes at 37 °C. ☞ Safe stopping point. After completing the reaction, the samples can be stored at -20 °C.
4	Add 16 µl RNase-free water to a total volume of 26 µl and insert the sample into step 2 of RiboCop rRNA depletion and continue with the protocol for CORALL FFPE RNA-Seq as decribed in 219UG781. Alternatively, purify the reaction using spin-column purification or magnetic beads suitable for RNA purification before proceeding to downstream reactions. For more information, please contact support@lexogen.com.