Important to Note:

For most downstream applications it is not necessary to do DNase digestion due to HiBind® RNA resin and spin column technology removing nearly all DNA without the need for DNase Treatment. However, certain sensitive RNA applications might require further removal of DNA. In such case, we recommend that you please follow the outlined steps below using product E1091.

1. Wash the HiBind® RNA column with RNA Wash Buffer I by pipetting250µl directly onto the spin column. Centrifuge at 10,000 x g for 1 minute, and discard the 2ml collecting tube.
2. For each HiBind® RNA column, prepare the DNase I stock solution as follows:
   E.Z.N.A.™ DNase I Digestion Buffer 73.5 µl
   RNase-free DNase I (20 Kunitz/:l) 1.5 µl
   Total Volume 75 µl
   NOTE: DNase I is very sensitive to physical denaturation, therefore do not vortex this DNase I mixture. Please mix by GENTLY inverting the tube. Remember to freshly prepare your DNase I stock solution right before RNA isolation.
   E.Z.N.A. ™ DNase I Digestion Buffer is supplied with Omega Bio-Tek, Inc.'s RNase-Free DNase Set (product no. E1091). Standard DNase Buffers are not compatible with on-membrane DNase digestion. The use of other buffers may affect the binding of RNA to the HiBind® matrix, reducing RNA yields, and purity.

3. Wash the HiBind® RNA column with RNA Wash Buffer I by pipetting 500µl directly onto the spin column. Centrifuge at 10,000 x g for 1 minute, and discard the 2ml collecting tube.
4. Pipet 75µl of the DNase I stock solution directly onto the surface of the

HiBind® RNA resin in each column (each column should be placed into a 2ml centrifuge tube). Make sure to pipet the stock solution directly onto the membrane. DNase I Digestion will not go through completion if some of the stock solution remains stuck to the wall or the o-ring of the HiBind® RNA column.

5. Incubate at room temperature (25-30°C) for 15 minutes.
6. Place the HiBind® RNA column into a new 2 ml centrifuge tube, and add 250µl of RNA Wash Buffer I. Place the column on a bench top for 2 minutes. Centrifuge at 10,000 x g for 1 minute and discard flow-through. Reuse the collection tube in step 7.
7. Place the HiBind® RNA column in the same 2 ml centrifuge tube, and add 500µl of RWB diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 minute and discard flow-through. Reuse the collection tube in step 8.
8. Wash the column with a second 500µl of RWB. Centrifuge and discard flow-through. Then, with the empty collection tube, centrifuge the HiBind® matrix for 2 minutes at maximum speed to completely dry the HiBind® matrix.
9. Place the column in a clean 1.5 ml microcentrifuge tube (not supplied), and add 40-70 µl of DEPC-treated water (supplied). Make sure to add water directly onto the HiBind® matrix. Let it sit for 1 minute, and then centrifuge for 2 minutes at 10,000 x g to elute the RNA. A second elution may be necessary if the expected yield of RNA > 30µg.

Alternatively, RNA may be eluted with a greater volume of water. While additional elutions increase Total RNA yield, the concentration will be lower since more than 80% of RNA has been recovered in the first elution. Preheating the water to 70°C before adding it to the column, and incubating the column for 5 min at room temperature before centrifugation may increase yields.