

**Plasma/Serum Circulating RNA Purification Kit (Slurry Format) –  
Supplementary Protocol for Optional On-Column DNA Removal  
Product # 42800**

Norgen's Plasma/Serum Circulating RNA Purification Kit (Slurry Format) isolates plasma/serum RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

1. For every on-column reaction to be performed, prepare a mix of 15  $\mu$ L of **DNase I** and 100  $\mu$ L of **Enzyme Incubation Buffer** using Norgen's RNase-Free DNase I Kit (Product # 25710). Mix gently by inverting the tube a few times. **DO NOT VORTEX**.

**Note:** If using an alternative DNase I, prepare a working stock of 0.25 Kunitz unit/ $\mu$ L RNase-free DNase I solution according to the manufacturer's instructions. A 100  $\mu$ L aliquot is required for each column to be treated.

2. Perform the appropriate Plasma/Serum Circulating RNA Purification Procedure for your starting material up to **Step 9**
3. Apply 400  $\mu$ L of **Wash Solution** to the column and centrifuge for **1 minute at 14,000 RPM**. Discard the flowthrough and reassemble the spin column with its collection tube.
4. Apply 100  $\mu$ L of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 14 000 RPM for 1 minute.

**Note:** Ensure that the entire DNase I solution passes through the column. If needed, spin at 14, 000 x g (~14 000 RPM) for an additional minute.

5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.

**Note:** Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

6. Incubate the column assembly at 25 - 30°C for 15 minutes.
7. Without any further centrifugation, proceed directly to the second wash step in **Step 11**.