NEXTFLEX® Small RNA-Seq Kit v3 tRNA/YRNA Depletion Protocol

The NEXTFLEX® Small RNA-Seq Kit v3 is compatible with cell-free RNA, such as RNA isolated from plasma. Users who wish to deplete the abundant tRNA fragments and Y RNA fragments found in many types of cell-free RNA should use the NEXTFLEX® tRNA/YRNA blocker (**not included in kit**). Please contact us at NGS@PerkinElmer.com if interested.

To use the NEXTFLEX® tRNA/YRNA Blocker, simply replace Step A with the following alternative step then proceed with the remainder of the protocol as written. (Please note that only Step A-1 differs from the standard protocol.)

Alternative STEP A: NEXTFLEX® 3' 4N Adenylated Adapter Ligation with tRNA/YRNA Blocker

1. For each sample, combine the following reagents on ice in nuclease-free 96-well PCR plate:

_ µL	RNA
_ µL	Nuclease-free Water
1 µL	NEXTFLEX® tRNA/YRNA Blocker
10.5 µL	TOTAL

2. Heat at 70°C for 2 minutes then immediately place on ice.
3. Incubate on ice for 2 – 5 minutes.
4. Note: Be sure to mix the following reaction until visibly homogenous by pipetting or brief vortexing. For each sample, combine the following reagents on ice in a nuclease-free 97-well PCR plate:

10.5 µL	Denatured RNA
1 µL	NEXTFLEX® 3' 4N Adenylated Adapter* (Up to 1/4 dilution may be used. See Table 1 in Starting Material)
7 µL	NEXTFLEX® 3' Ligation Buffer
1.5 µL	NEXTFLEX® 3' Ligation Enzyme Mix
20 µL	TOTAL

5. Mix thoroughly by pipetting.
6. Incubate at 25°C for 2 hours in a thermal cycler with heated lid turned off or left open. Incubation overnight at 20C may increase yield in some cases. For ligations to 2' O-methylated small RNAs, such as those found in plants, incubate at 16°C overnight.
7. Proceed immediately to Step B: Excess 3' Adapter Removal per standard protocol.