Polaris Depletion Kit – rRNA/Globin (HMR)

Product Description

Ribosomal RNA (rRNA) and globin mRNA are highly abundant in human, mouse, and rat total RNA. To improve the detection of RNA of interest, the Polaris Depletion Kit – rRNA/Globin (HMR) specifically targets and efficiently depletes:

- 28S, 18S, 5.8S, and 5S cytoplasmic rRNAs
- 16S and 12S mitochondrial rRNAs
- 45S ETS and ITS rRNAs (probes designed for human only)
- HBA1/2, HBB, HBD, HBM, HBG1/2, HBE1, HBQ1, and HBZ globin RNAs (probes designed for human only)

Product Applications

The Polaris Depletion Kit – rRNA/Globin (HMR) is ideally suited for:

- Processing both intact and degraded RNA, including FFPE
- Processing human, mouse, and rat RNA
- rRNA and globin mRNA depletion upstream of RNA library preparation. If using the Polaris Depletion Kit upstream of the Watchmaker RNA Library Prep Kit, please refer to WMUG210 for a combined protocol.

Table of Contents

Kit Contents

Kit	Kit Code	Description	Component Volume	
			24 rxn	96 rxn
Polaris Depletion Kit – rRNA/Globin (HMR)	**7K0077-024 (24 rxn)** **7K0077-096 (96 rxn)**	Depletion Master Mix	120 µL	540 µL
		Depletion Probes – rRNA/Globin (HMR)	70 µL	300 µL
		Probe Digestion Master Mix	925 µL	4.2 mL

For custom formats, contact the **Sales Team** at sales@watchmakergenomics.com.

Storage and Handling

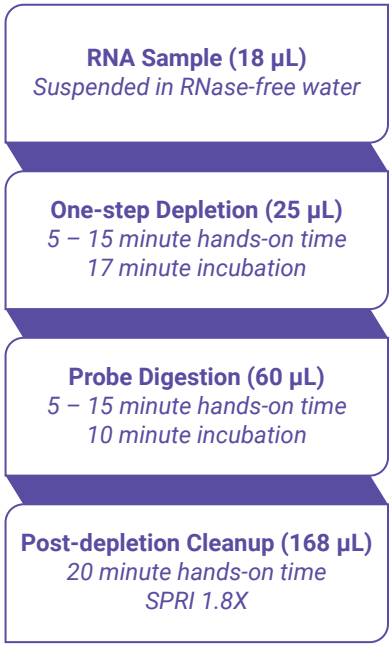
The Polaris Depletion Kit – rRNA/Globin (HMR) is shipped on cold packs. Upon receipt, store all components at -20 ± 5°C. Keep all components and reaction mixes on ice or a cooled reagent block during routine use, unless otherwise stated. Some components are viscous; therefore take care to homogenize solutions thoroughly before use and during reaction setup. SPRI beads should be handled as per the manufacturer's guidelines. All buffers should be vortexed for at least 5 sec before use. All master mixes (buffer and enzyme combined) prepared in the protocol should be stored at 4˚C unless stated otherwise.

Required Materials Not Included

- Magnetic rack compatible with 0.2 mL PCR tubes and/or 96-well plate
- Ampure® XP Beads (Beckman Coulter, Inc. #A63881) or equivalent
- 80% Ethanol
- 200 µL thin-wall PCR tubes compatible with thermocycler, or 96-well 0.2 mL PCR Plates and Plate-Seal
- 1 mL, 2 mL, 5 mL tubes (RNase-free)
- RNase-free water
- 10 mM Tris-HCl, pH 8.0
- Thermocycler
- Fragment Analyzer™ e.g., Bioanalyzer® or TapeStation (Agilent Technologies, Inc.), or similar instrument and consumables

These protocols are designed for use with the specified labware, consumables, and calibrated equipment.

Workflow Overview



Prior to Starting

Selecting the appropriate protocol for use

This protocol is intended to provide instructions for the use of the Polaris Depletion Kit – rRNA/Globin (HMR). If using the kit upstream of the Watchmaker RNA Library Prep Kit, refer to WMUG210 for a combined protocol.

Input RNA quality and quantity

This kit is compatible with both high- and low-quality, including FFPE and blood-derived, samples suspended in 18 µL of RNase-free water.

RNA should be accurately quantified by Qubit® Fluorometer or similar prior to starting.

High-quality total RNA ranging from 1 – 1000 ng has been tested and shown successful depletion.

Input RNA Purity

RNA inputs should be free from contaminating DNA. If the total RNA contains DNA, remove the contamination by incubating with DNase I (not supplied with kit). Residual DNase I may interfere with library preparation, so it is important to ensure no residual enzyme remains in the sample.

RNA should be suspended in RNase-free water and be free of salts (e.g., Mg^{2+}, or guanidinium salts), chelating agents (e.g., EDTA or EGTA), and organics (e.g., phenol or ethanol).

RNA Handling

To avoid RNase contamination, work in a laminar flow hood, if available, and keep all sample and reagent tubes closed unless in use. Wear gloves when handling reagents and preparing libraries. Change gloves and pipette tips if they come into contact with non-sterile surfaces. To avoid RNA degradation, store RNA in an RNase-free diluent and limit the number of sample freeze-thaw cycles.

SPRI Purification Beads

The protocol outlined below assumes the use of AMPure® XP (Beckman Coulter) reagent for bead purification steps.

Other SPRI bead brands may be used, assuming they are nuclease-free and deliver equivalent sizing performance at the given bead ratios. Otherwise, bead purification ratios will need to be optimized for the bead brand used.

Ensure beads are equilibrated to room temperature and thoroughly resuspended via vortexing prior to use.

Protocol

Recommendations

- If using the kit upstream of the Watchmaker RNA Library Prep Kit, refer to WMUG210 for a combined protocol.
- Keep all sample tubes, buffers, and enzymes on ice unless stated otherwise.
- Vortex mixing is recommended for master mix generation and subsequent addition to sample. Pipette mixing is an acceptable alternative so long as care is taken to ensure a completely homogeneous reaction.
- Ensure all components are fully thawed on ice before use. Once thawed, invert the tubes several times, and vortex for at least 5 sec to ensure the reagent is fully mixed.
- Where possible, centrifuge briefly to remove any excess liquid from the tube and collect all liquid from the tube lids prior to opening a tube.
- We recommend making master mixes for each reaction step with a 10% excess to account for loss during pipetting.
- Ensure SPRI purification beads are fully equilibrated to room temperature and thoroughly resuspended by vortexing prior to use.

1. rRNA and Globin Depletion

1.1 Program a thermocycler as indicated below and initiate the run to heat the block:

Step	Temperature	Time
Lid temperature	80°C	N/A
HOLD	77°C	HOLD
Denaturation	77°C	2 min
rRNA digestion	65°C	15 min
HOLD	4°C	HOLD

1.2 For each reaction, prepare the Depletion Reaction Mix as specified below on ice:

Component	Volume (µL)
Depletion Master Mix	4.5
Depletion Probes – rRNA/Globin (HMR)	2.5

1.3 Mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.

1.4 On ice, prepare input RNA in a total volume of 18 µL using RNase-free water and add to labeled 0.2 mL PCR tubes or PCR plate.

1.5 To each sample, add the following on ice:

Component	Volume (µL)
RNA sample	18
Depletion Reaction Mix	7

1.6 Mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.

1.7 Place the tube on ice until ready for thermocycling.

NOTE: Samples should remain on ice for no longer than 10 min.

1.8 Place the tube into the heated thermocycler (programmed and initiated in **Step 1.1**). Advance the thermocycler from the initial 77°C hold.

1.9 Upon completion of thermocycling, proceed immediately to **Probe Digestion**.

2. Probe Digestion

2.1 Program a thermocycler as indicated below and initiate the run to cool the block:

Step	Temperature	Time
Lid temperature	40°C	N/A
Pre-cooling	4°C	HOLD
Probe digestion	37°C	10 min
HOLD	4°C	HOLD

2.2 To each sample, add the Probe Digestion Master Mix as specified below on ice:

Component	Volume (µL)
Depleted RNA	25
Probe Digestion Master Mix	35

2.3 Mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.

2.4 Place the tube in the chilled thermocycler (programmed and initiated in **Step 2.1**). Advance the thermocycler from the initial 4°C hold to start the 37°C incubation.

2.5 Upon completion of the Probe Digestion incubation, proceed immediately to **Post-depletion Cleanup**.

3. Post-depletion Cleanup

3.1 Freshly prepare at least 0.4 mL of an 80% ethanol solution for each reaction.

3.2 Vortex **room temperature** SPRI beads to thoroughly mix. Add 108 µL (1.8X) of beads to each reaction.

3.3 Mix on a touch vortexer (or 3,000 rpm) for 4 sec. Briefly centrifuge to collect all liquid in the bottom of the tube.

3.4 Incubate the reaction-bead mixtures at room temperature for 5 min.

3.5 Place sample tubes on a magnet for at least 5 min, or until all beads have collected on the tube wall and the solution is clear.

3.6 Carefully remove and discard the supernatant from each tube.

3.7 Add 200 µL of freshly prepared 80% ethanol to each tube, taking care to not disturb the bead pellet on the tube wall.

3.8 Incubate tubes at room temperature for at least 30 sec without removing the tube from the magnet or disturbing the bead pellet. Carefully remove and discard the ethanol.

3.9 Repeat **Steps 3.7 – 3.8**, for a total of two washes.

OPTIONAL: Tubes can be briefly spun to pull down excess ethanol prior to removing with a p10 pipette.

3.10 Allow remaining ethanol to evaporate by allowing the pellets to air dry for 3 – 5 min.

NOTE: Beads have been sufficiently dried when cracks appear in the surface of the bead pellet and no liquid is visible in the tube.

3.11 Carefully resuspend each bead pellet in 22 µL of RNase-free water.

NOTE: If planning to proceed into the Watchmaker RNA Library Prep Kit, skip this step and refer immediately to WMUG210.

3.12 Incubate tubes at room temperature for at least 2 min before placing back on the magnet.

3.13 Leave tubes on the magnet for at least 2 min, or until all beads have collected on the tube wall and the solution is clear.

3.14 Carefully transfer 20 µL to a new, labeled tube.

NOTE: RNA is unstable and prone to degradation over time and freeze-thaw cycling. Ensure proper storage to maintain integrity.

For Technical Support, please contact the **Scientific Support Team** at support@watchmakergenomics.com.

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PTD-26 WMUG211