KAPABIOSYSTEMS

Technical Data Sheet

KAPA RiboErase Kit (HMR)

Human/Mouse/Rat

KR1142 – v2.16

This Technical Data Sheet provides product information and a detailed protocol for the KAPA RiboErase Kit (HMR or Human/Mouse/Rat).

Contents

Kapa/*Roche* Kit Codes and Components		
KK8481 *07962266001* 24 reactions	Hybridization Buffer	110 µL
	Hybridization Oligos (HMR)	110 µL
	Depletion Buffer	80 µL
	RNase H	55 µL
	DNase Buffer	60 µL
	DNase	55 µL
KK8482 *07962274001* 96 reactions	Hybridization Buffer	480 µL
	Hybridization Oligos (HMR)	480 µL
	Depletion Buffer	360 µL
	RNase H	240 µL
	DNase Buffer	264 µL
	DNase	240 µL

Quick Notes

- This protocol is suitable for the depletion of ribosomal RNA from 100 ng – 1 µg of total human, mouse, or rat RNA (HMR).
- Suitable for high- and low-quality RNA samples, including FFPE. Results may vary depending on the input amount and quality.
- Removes >99% of ribosomal RNA, enriching for mature and immature mRNAs, and long noncoding RNAs.
- We recommend the use of KAPA Pure Beads (KK8000, KK8001, KK8002) for cleanup steps. KAPA Pure Beads are sold separately.

Product Description

The KAPA RiboErase Kit (Human/Mouse/Rat or HMR) contains all of the buffers and enzymes required for depletion of ribosomal RNA (rRNA) from 100 ng – 1 µg of total RNA via the following steps:

1. hybridization of DNA oligonucleotides complementary to rRNA of human, mouse, and rat species;
2. RNase H treatment to remove rRNA duplexed to DNA oligonucleotides; and
3. DNase treatment to remove original DNA oligonucleotides.

The kit provides all of the enzymes and buffers required for rRNA depletion, but does not include RNA, RNase-free water, or beads for cleanup steps.

Product Applications

The KAPA RiboErase Kit (HMR) is designed for both manual and automated depletion of rRNA from 100 ng – 1 µg of total RNA. The kit depletes both cytoplasmic (5S, 5.8S, 18S, and 28S) and mitochondrial (12S and 16S) rRNA species.

Product Specifications

Shipping and Storage

The enzymes provided in this kit are temperature sensitive, and appropriate care should be taken during shipping and storage. KAPA RiboErase Kits (HMR) are shipped on dry ice or ice packs, depending on the destination country. Upon receipt, immediately store enzymes and reaction buffer components at -15°C to -25°C in a constant temperature freezer. When stored under these conditions and handled correctly, the kit components will retain full activity until the expiry date indicated on the kit label.

Handling

Always ensure that components have been fully thawed and thoroughly mixed before use. Keep all enzyme components and master mixes on ice whenever possible during handling and preparation, unless specified otherwise.

Quality Control

All kit components are subjected to stringent functional quality control, are free of detectable contaminating exo- and endonuclease activities, and meet strict requirements with respect to DNA contamination. Please contact Technical Support at **kapabiosystems.com/support** for more information.

Important Parameters

Input RNA Requirements

- This protocol has been validated for rRNA depletion from 100 ng – 1 µg total RNA, in 10 µL of RNase-free water.
- The quantity of rRNA in a total RNA sample can vary significantly between samples. If library preparation is to be performed downstream, an input of 100 ng - 1 µg of total RNA is recommended to ensure sufficient rRNA-depleted RNA is available.
- RNA in volumes >10 µL should be concentrated to 10 µL prior to use by ethanol precipitation, bead purification (e.g., KAPA Pure Beads or RNAClean® XP, Beckman Coulter®), or column-based methods (e.g., RNeasy® MinElute® Cleanup Kit, QIAGEN). Note that some loss of material is inevitable when using any of the above methods to concentrate RNA.
- When concentrating RNA, an elution volume of 12 µL of RNase-free water is recommended to ensure that 10 µL is available for use in this protocol.
- It is recommended to assess the quality and size distribution of the input RNA prior to rRNA depletion by electrophoretic method (e.g., Agilent Bioanalyzer RNA assay).

RNA Handling

- RNases are ubiquitous and special care should be taken throughout the procedure to avoid RNase contamination.
- To avoid airborne RNase contamination, keep all reagents and RNA samples closed when not in use.
- Use a laminar flow hood if available, or prepare a sterile and RNase-free area. Clean the workspace, pipettes, and other equipment with an RNase removal product (e.g., RNase*Zap*®, Ambion® Inc.) according to manufacturer's recommendations.
- To avoid RNase contamination, always wear gloves when handling reagents, and use certified RNase-free plastic consumables. Change gloves after making contact with equipment or surfaces outside of the RNase-free working area.
- To mix samples containing RNA, gently pipette the reaction mixture several times. Vortexing may fragment the RNA, resulting in lower quantity and a reduced library insert size.
- To avoid degradation, minimize the number of freeze-thaw cycles and always store RNA in RNase-free water.

Reaction Setup

This kit is intended for manual and automated depletion of ribsomal RNA from human, mouse, and rat samples. To ensure optimal performance, reaction components should be combined into master mixes, rather than dispensed separately into individual reactions. When processing multiple samples, prepare a minimum of 10% excess of each master mix to allow for small inaccuracies during dispensing. Recommended volumes for 8, 24, and 96 reactions (with excess) are provided in Tables 1 – 3.

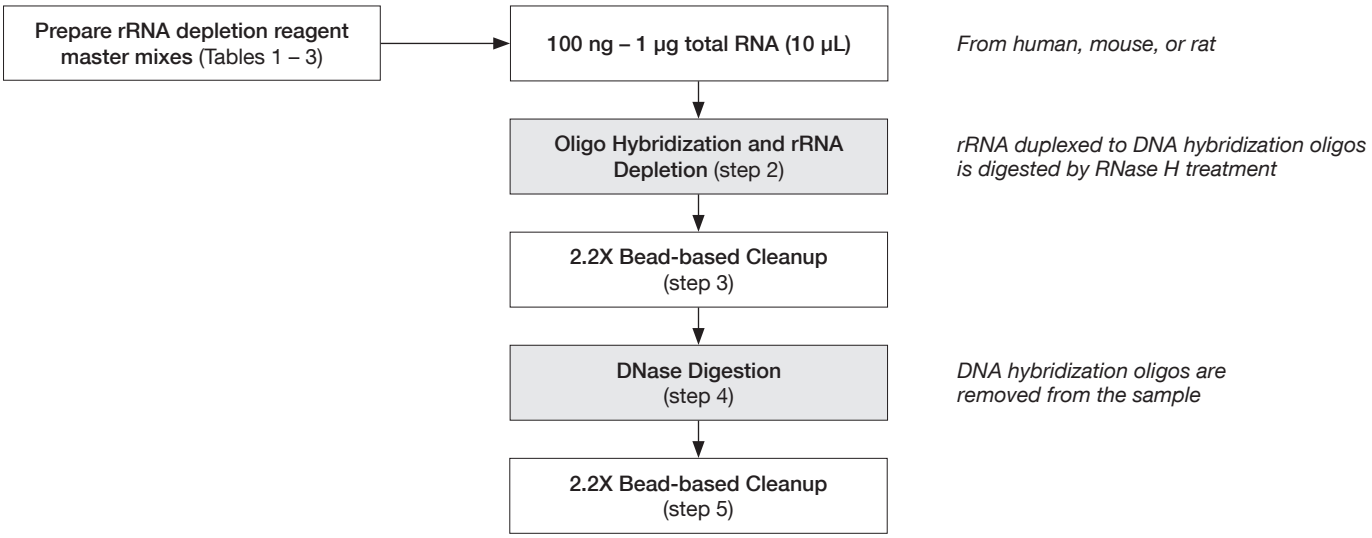
Depletion reactions may be prepared in standard reaction vessels, including 1.5 mL microtubes, PCR tubes, strip tubes, or PCR plates. Always use plastics that are certified to be RNase- and DNase-free. Low RNA- and DNA-binding plastics are recommended. When selecting the most appropriate plastic consumables for your workflow, consider compatibility with:

- the magnet used during bead manipulations;
- vortex mixers and centrifuges, where appropriate; and
- Peltier devices or thermocyclers used for reaction incubations.

Reaction Cleanups

- This protocol has been validated for use with either KAPA Pure Beads (KK8000, KK8001, KK8002) or using Agencourt® AMPure® XP (Beckman Coulter®). Solutions and conditions for RNA binding may differ if other beads are used.
- Cleanup steps should be performed in a timely manner to ensure that enzymatic reactions do not proceed beyond optimal incubation times.
- Observe the storage and handling recommendations for KAPA Pure Beads or Agencourt AMPure XP. Equilibration at room temperature is essential to achieve specified size distribution and yield of libraries.
- Beads will settle gradually; ensure that they are fully resuspended before use.
- ***To ensure optimal RNA recovery, it is critical that the RNA and KAPA Pure Beads are thoroughly mixed*** (by vortexing or extensive up-and-down pipetting) before the RNA binding incubation.
- Bead incubation times are guidelines only, and may be modified/optimized according to current protocols, previous experience, specific equipment, and samples in order to maximize library construction efficiency and throughput.
- The time required for complete capture of beads varies according to the reaction vessel and magnet used. It is important not to discard or transfer any beads with the removal of the supernatant. Capture times should be optimized accordingly.
- The volumes of 80% ethanol used for the bead washes may be adjusted to accommodate smaller reaction vessels and/or limited pipetting capacity, but always ensure that the beads are entirely submerged during the wash steps. ***Always use freshly prepared 80% ethanol.***
- It is important to remove all ethanol before proceeding with subsequent reactions. However, over-drying of beads may make them difficult to resuspend, and result in a dramatic loss of RNA. With optimized aspiration of ethanol, drying of beads for 3 – 5 min at room temperature should be sufficient. ***Drying of beads at 37°C is not recommended.***
- Where appropriate, RNA should be eluted from beads in RNase-free water.

Process Workflow



Ribosomal RNA Depletion Protocol

1. Reagent Preparation

For maximum stability and shelf-life, enzyme and reaction buffers are supplied separately in the KAPA RiboErase Kit (HMR).

In the examples given in the following tables, all of the required water is included in each master mix, allowing the entire reaction mix to be added in a single pipetting step. Additional reagents required for the KAPA RiboErase protocol are listed in Table 4.

Always ensure that KAPA Pure Beads are fully equilibrated to room temperature before use.

Table 1. Oligo hybridization

Component:	1 library	8 libraries *Inc. 10% excess*	24 libraries *Inc. 10% excess*	96 libraries *Inc. 10% excess*
Hybridization master mix				
Hybridization Buffer	4 µL	35.2 µL	106 µL	423 µL
Hybridization Oligos (HMR)	4 µL	35.2 µL	106 µL	423 µL
RNase-free water	2 µL	17.6 µL	53 µL	211 µL
Total master mix volume:	**10 µL**	**88 µL**	**265 µL**	**1057 µL**
Final reaction composition:				
Hybridization master mix	10 µL			
Total RNA	10 µL			
Total reaction volume:	**20 µL**			

Table 2. rRNA depletion

Component:	1 library	8 libraries *Inc. 10% excess*	24 libraries *Inc. 10% excess*	96 libraries *Inc. 10% excess*
Depletion master mix				
Depletion Buffer	3 µL	26.4 µL	80 µL	317 µL
RNase H	2 µL	17.6 µL	53 µL	211 µL
Total master mix volume:	**5 µL**	**44.0 µL**	**133 µL**	**528 µL**
Final reaction composition:				
Depletion master mix	5 µL			
Total RNA hybridized to oligos	20 µL			
Total reaction volume:	**25 µL**			

Table 3. DNase digestion

Component:	1 library	8 libraries *Inc. 10% excess*	24 libraries *Inc. 10% excess*	96 libraries *Inc. 10% excess*
DNase digestion master mix				
DNase Buffer	2.2 µL	19.4 µL	58 µL	232 µL
DNase	2 µL	17.6 µL	53 µL	211 µL
RNase-free water	17.8 µL	157 µL	470 µL	1880 µL
Total master mix volume:	**22 µL**	**194 µL**	**581 µL**	**2323 µL**
Resuspend beads in a volume of:	**22 µL**			

Table 4. Volumes of additional reagents required

Component:	1 library	8 libraries *Inc. 10% excess*	24 libraries *Inc. 10% excess*	96 libraries *Inc. 10% excess*
KAPA Pure Beads (sold separately)	100 µL	880 µL	3 mL	11 mL
80% ethanol (freshly prepared; not supplied)	800 µL	7 mL	22 mL	85 mL

2. Oligo Hybridization and rRNA Depletion

This protocol requires 100 ng – 1 µg of total RNA, in 10 µL of RNase-free water.

Ensure that the hybridization master mix (Table 1) and the depletion master mix (Table 2) are prepared and kept at room temperature before use.

2.1 Program a thermocycler as follows:

Step	Temp.	Duration
Hybridization	95°C	2 min
Ramp down to 45°C at -0.1°C/s		
PAUSE	45°C	∞
Depletion	45°C	30 min
HOLD	4°C	∞

2.2 Assemble rRNA hybridization reactions as follows:

Component	Volume
Total RNA in water	10 µL
Hybridization master mix at room temperature (Table 1)	10 µL
Total volume:	**20 µL**

2.3 Place samples in the pre-programmed thermocycler and execute the program.

2.4 Ensure the depletion master mix containing RNase H is added while the samples are kept at 45°C in a thermocycler. When the program reaches the pause step at 45°C, add the following to each 20 µL hybridization reaction.

Component	Volume
Depletion master mix at room temperature (Table 2)	5 µL
Total volume:	**25 µL**

2.5 Resume the cycling program to continue with the depletion step (45°C for 30 min).

2.6 Proceed immediately to **rRNA Depletion Cleanup** (Step 3)

3. rRNA Depletion Cleanup

3.1 Perform a 2.2X bead-based cleanup by combining the following:

Component	Volume
rRNA-depleted sample	25 µL
KAPA Pure Beads	55 µL
Total volume:	**80 µL**

3.2 Thoroughly resuspend the beads by pipetting up and down multiple times.

3.3 Incubate the plate/tube(s) at room temperature for 5 min to bind the RNA to the beads.

3.4 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

3.5 Carefully remove and discard 75 µL of supernatant.

3.6 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

3.7 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

3.8 Carefully remove and discard the ethanol.

3.9 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

3.10 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

3.11 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.

3.12 Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. ***Caution: over-drying the beads may result in reduced yield.***

4. DNase Digestion

To remove the hybridization oligo-nucleotides, the ribosomal depleted sample is incubated with DNase. ***Ensure that the DNase digestion master mix (Table 3) is prepared and kept at room temperature.***

4.1 Assemble DNase digestion reactions as follows:

Component	Volume
Beads with rRNA-depleted sample	–
DNase digestion master mix at room temperature (Table 3)	22 µL
Total volume:	**22 µL**

4.2 Thoroughly resuspend the beads by pipetting up and down multiple times.

4.3 Incubate the plate/tube(s) at room temperature for 3 min to elute the RNA off the beads.

4.4 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

4.5 Carefully transfer 20 µL of supernatant into a new plate/tube(s). Discard the plate/tube(s) with beads.

4.6 Incubate the plate/tube(s) with supernatant using the following protocol:

Step	Temp.	Duration
DNase digestion	37°C	30 min
HOLD	4°C	∞

4.7 Proceed immediately to **DNase Digestion Cleanup** (step 5).

5. DNase Digestion Cleanup

5.1 Perform a 2.2X bead-based cleanup by combining the following:

Component	Volume
DNase-treated RNA	20 µL
KAPA Pure Beads	44 µL
Total volume:	**64 µL**

5.2 Thoroughly resuspend the beads by pipetting up and down multiple times.

5.3 Incubate the plate/tube(s) at room temperature for 5 min to bind the RNA to the beads.

5.4 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

5.5 Carefully remove and discard 60 µL of supernatant.

5.6 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

5.7 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

5.8 Carefully remove and discard the ethanol.

5.9 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.

5.10 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.

5.11 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.

5.12 Dry the beads at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. ***Caution: over-drying the beads may result in reduced yield.***

5.13 Thoroughly resuspend the beads (with purified, DNase-treated RNA) in an appropriate volume of RNase-free water by pipetting up and down multiple times.

5.14 Incubate the plate/tube(s) at room temperature for 3 min to elute RNA off the beads.

5.15 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.

5.16 Carefully transfer the appropriate volume of supernatant into a new plate/tube(s). Discard the plate/tube(s) with beads. Store rRNA-depleted samples at -80ºC.

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