Liquid Biopsy Research - “We are where you start”

HighPrep™ Total RNA Kit

Manual Revision v1.01
Catalog Nos. HPTR-R12, HPTR-R96, HPTR-R96X4

- RNA isolation from 200 µl lysate of tissues, mouse tails, cultured cells, or buccal swabs
- Magnetic beads based chemistry

Contents

Product Description and Process.. 1
Kit Contents, Storage, Stability.. 1
Preparation of Reagents 2
HighPrep™ Total RNA - Tissue protocol.............................. 3
Troubleshooting Guide 6

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Product Description

The HighPrep™ Total RNA kit is a paramagnetic beads based system that allows a fast and efficient purification of high-quality total RNA from tissues and cultured cells suitable for numerous high performance RNA downstream applications such as RT-PCR, Microarrays, cDNA synthesis. As a paramagnetic based system, the HighPrep™ Tissue RNA kit can be used in a high-throughput format, hence adaptable to numerous commercially available workastations.

Process

The HighPrep™ Tissue RNA kit uses a simple 3 steps procedure: Lyse+Bind-Wash-Elute. Tissue or cells are lysed and released DNA and RNA binds to the HighPrep™ Mag-R1 magnetic beads in one step. Utilizing a magnetic separation device, the bound genomic DNA and RNA, separated from the solution is washed. Genomic DNA is removed with a DNase Digestion step. After 2 wash steps, RNA is eluted from magnetic beads.

Kit Contents and Storage

HighPrep™ Total RNA Kits Catalog No.	HPTR-R12	HPTR-R96	HPTR-R96X4	STORAGE
Number of Preps	12	96	384	
RNA Lysis Buffer	6 mL	50 mL	200 mL	15-25°C
HSW Buffer[1]	3 mL	22 mL	88 mL	15-25°C
Pro K Solution	300 µL	3 mL	12 mL	2-8°C
DNase	30 µL	220 µL	4 x 220 µL	-20°C
DNase Digestion Buffer	2 mL	12 mL	48 mL	15-25°C
RNA Elution Buffer	650 µL	5 mL	20 mL	15-25°C
MAG-R1 Particles	300 µL	2.2 mL	8.8 mL	2-8°C

[1] Ethanol must be added prior to use. See Preparation of Reagents

Stability

All components are stable for 12 months when stored accordingly.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Resource" tab when viewing the product kit.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add 100% Isopropanol	Add 100% Ethanol	Storage
HPTR-R12	**HSW Buffer**	--------------	3.8 mL	Room Temp 15-25°C
Components are stable for 1 year when stored closed at room temperature				

Catalog No.	Component	Add 100% Isopropanol	Add 100% Ethanol	Storage
HPTR-R96	**HSW Buffer**	--------------	28 ml	Room Temp 15-25°C
Components are stable for 1 year when stored closed at room temperature				

Catalog No.	Component	Add 100% Isopropanol	Add 100% Ethanol	Storage
HPTR-R96X4	**HSW Buffer**	--------------	112 ml	Room Temp 15-25°C
Components are stable for 1 year when stored closed at room temperature				

Preparation of DNase Mix (100µl):

Component	Amount per Prep
DNase Digestion Buffer	98 µl
DNase	2 µl

HighPrep™ Total RNA - Tissue - 96 well format protocol

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Centrifuge capable of 4,000 x g with swing bucket rotor for 96 well plates
- ☐ Equipment for disrupting tissue or mortar and pestle
- ☐ Liquid nitrogen (if using mortar and pesltle method)
- ☐ Vortexer
- ☐ 96 magnetic separation device for deep well plate
- ☐ Multi-channel pipettor and nuclease-free pipette tips
- ☐ Multi-channel reservoirs
- ☐ Sealing film for storage
- ☐ 96-well microplates with minimum capacity of 1 ml
- ☐ 100% Ethanol

Things to do before starting

- ☐ Ensure HSW Buffer are prepared according to the instructions on page 2 and are at room temperature.
- ☐ If any of the buffers contained precipitates during storage, warm solution at 45°C.
- ☐ Shake or vortex thoroughly the MAG-R1 magnetic particles to fullly resuspend.

Protocol - 96 Well Plate and 1.5 ml tube format

1. **Prepare the 96 well deep plate by adding 450 µL RNA Lysis Buffer to each sample well. (or in a 1.5 mL centrifuge tube if dealing with only a few samples)**

2. **Weigh up to 10mg tissue sample.**

3. **Mince tissue samples in small pieces using 2 razor blades**

4. **Disruption and homogenization of tissue sample.** It is essential to to use effective disruption method to acheive isolation of quality RNA. **Choose one of the following:**

Commercially avaliable homogenizers such as the Omni Polytron or the GenoGrider 2000,2010 may be used.

A. Tissue sample disruption using Grinder Method

i. Place minced sample tissue into a stainless steel grinding container with steel beads. Add 450 µL RNA Lysis Buffer to each sample well. Grind sample at 15-20 second intervals resting for 5 seconds between each interval for a total of 120 seconds. During the homogenization, set the grinder to half-speed as this will sufficiently disrupt the tissue without producing foam.

ii. Remove the plate from the homogenizer and remove the caps.
Note: It may be necessary to centrifuge the plate briefly to remove debris from the caps.

B. Manual tissue sample preparation:

i. Placed minced tissue sample tissue into a 30 mL mortar.
ii. Add liquid nitrogen to freeze the tissue pieces.
iii. Grind frozen tissue pieces until a ground powder is obtained.
iv. Pour ground tissues into a sample well (or in a 1.5 mL centrifuge tube if dealing with only a few samples) containing 450µL of RNA Lysis Buffer

1. Note: Place the frozen tissue directly into the Lysis Buffer before it begins to thaw to avoid RNA degradation.

5. **Once sample preparation or hogenization is completed, Add 20µl Pro K Solution to the samples and mix by pippetting 20 times up and down or by vortexing for 15 s.**
   1. Note: Homogenization time may vary with tissue type: Fibrous tissue may 5 minutes at the highest speed setting.

6. **Centrifuge the sample plate at 4,000 x g for 5 min.**

7. **Transfer 400 µl homogenized lysate to a new 96-well processing plate or fresh tube.**

8. **Add 300 µL 100% ethanol and 20 µL MAG-R1 particles to each sample. Pipette mix 10 times. Incubate at room temperature for 5 min.**

   ⚠ Complete resuspension of the HighPrep™ Mag-R1 particles is crucial for obtaining high purity.

9. **Place the sample plate on the magnetic separation device for 4 min or until the magnetic beads clear from solution.**

10. **With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**
    ⚠ Do not disturb the attracted beads while aspirating the supernatant.

11. **Remove plate or tube from the magnet and wash by adding 400 µl HSW Buffer to sample buffer and gently pipette mix 5 times.**

    ⚠ Shake thoroughly the HighPrep™ Mag-C1 particles to fully resuspend before use.

12. **Return the sample plate back on the magnetic separation device for 4 minsutes or until the magnetic beads clear from solution before proceeding to next step.**

13. **With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**
    ⚠ Do not disturb the attracted beads while aspirating the supernatant.

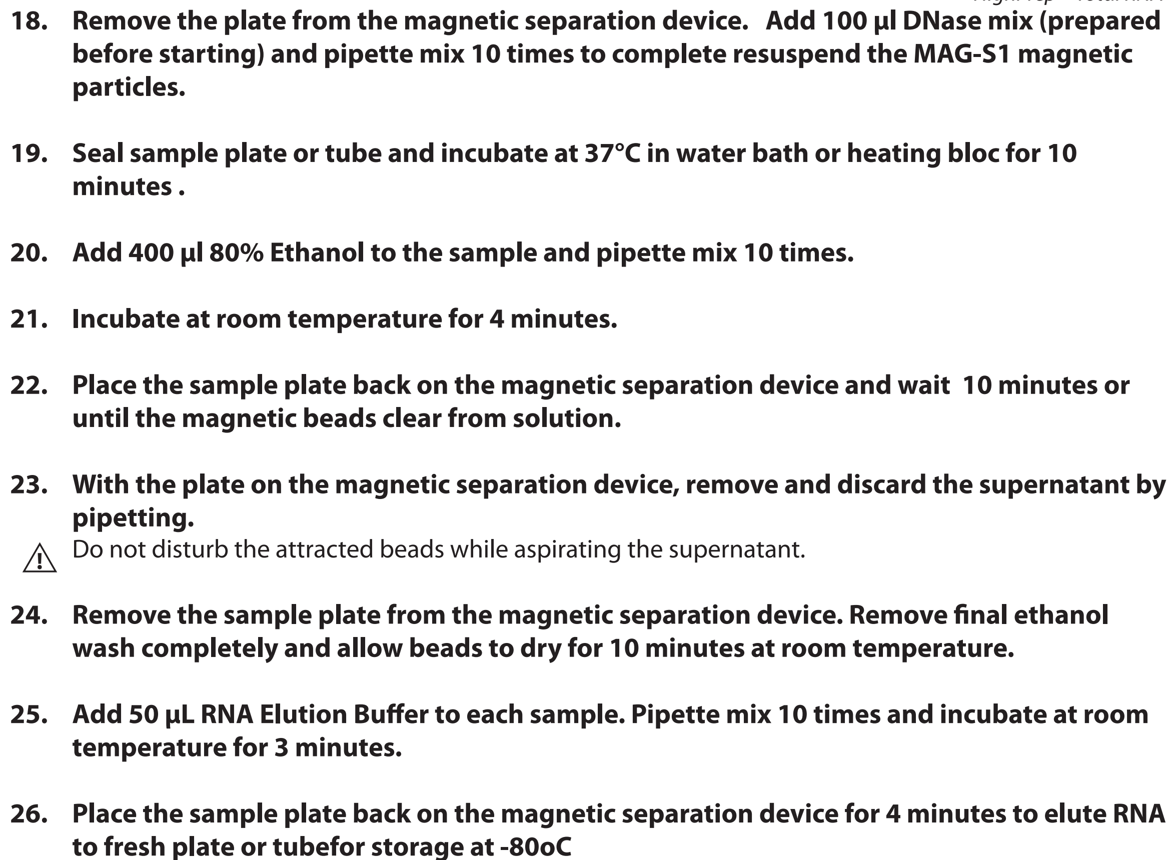
14. **Remove the plate from the magnetic separation device. Add 400 µl 80% Ethanol to the sample and pipette mix 10 times.**

    ⚠ Complete resuspension of the HighPrep™ Mag-C1 particles is crucial for obtaining high purity.

15. **Place the sample plate back on the magnetic separation device for 4 minutes or until the magnetic beads clear from solution.**

16. **With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**
    ⚠ Do not disturb the attracted beads while aspirating the supernatant. Remove as much Wash buffer as possible, then REMOVE the plate or tube from the magnet.

17. **Dry the beads by incubating for 4 minutes at room temperature with the plate still on the magnetic separation device.**



18. **Remove the plate from the magnetic separation device. Add 100 µl DNase mix (prepared before starting) and pipette mix 10 times to complete resuspend the MAG-S1 magnetic particles.**

19. **Seal sample plate or tube and incubate at 37°C in water bath or heating bloc for 10 minutes .**

20. **Add 400 µl 80% Ethanol to the sample and pipette mix 10 times.**

21. **Incubate at room temperature for 4 minutes.**

22. **Place the sample plate back on the magnetic separation device and wait 10 minutes or until the magnetic beads clear from solution.**

23. **With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**

    ⚠ Do not disturb the attracted beads while aspirating the supernatant.

24. **Remove the sample plate from the magnetic separation device. Remove final ethanol wash completely and allow beads to dry for 10 minutes at room temperature.**

25. **Add 50 µL RNA Elution Buffer to each sample. Pipette mix 10 times and incubate at room temperature for 3 minutes.**

26. **Place the sample plate back on the magnetic separation device for 4 minutes to elute RNA to fresh plate or tubefor storage at -80oC**

Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: 1-855-262-4246 (in US), outside US, 1-919-719-0665
Email: support@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low DNA yield	Incomplete resuspension of HighPrep™ Mag-C1 particles	Resuspend the HighPrep™ Mag-C1 particles by vortexing before use.
	RNA degraded during storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	Low levels of leukocytes	Low white blood cells count will give reduced yield.
	Incomplete resuspension of HighPrep™ Mag-C1 particles	Resuspend HighPrep™ Mag-C1 particles by vortexing vigorously before use.
	RNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	Loss of HighPrep™ Mag-C1 particles during procedure	Be careful not to remove the HighPrep™ Mag-C1 particles during the procedure.
	Ethanol was not added to RNA Wash Buffer II	Add ethanol to RNA Wash Buffer II as instructed on Page 4.
	HighPrep™ Mag-C1 particles not resuspended during binding	Vortex vigorously for 2 minutes after addition of ethanol and HighPrep™ Mag-C1 particles
Problem with downstream application	Insufficient RNA was used	IRNA in the sample already degraded. Do not freeze/thaw the sample more than once. Do not store at room temperature.
Carryover of the 15-25°C in the elution	Carryover of the HighPrep™ Mag-C1 particles in the eluted RNA will not effect downstream applications.	To remove the carryover HighPrep™ Mag-C1 particles from the eluted RNA, simply place the plate on the magnetic separation device and wait until the eluate has cleared. Carefully transfer the RNA eluate to a new 96-well plate.

Ordering Information

Product Description	Catalog No.	Preps
HighPrep™ Total RNA kit - 96 preps	HPTR-R96	96
HighPrep™ Total RNA kit - 384 preps	HPTR-R396	384

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