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HighPrep™ Total RNA Plus Kit

Catalog Nos. HPTOR-R5, HPTOR-R50, HPTOR-R100, HPTOR-R100X4
Manual Revision 2.1
WI-72-28

- RNA isolation from tissues, cultured cells, and whole blood
- Magnetic bead-based chemistry

Contents

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

The HighPrep™ Total RNA Plus Kit is specially designed for purifying total RNA from a wide variety of tissues, cultured cells, whole blood, and buccal swabs. The kit uses the special lysis condition with HighPrep™ magnetic particles technology to isolate high-quality total RNA from 5-30 mg of tissue, 200 µl of whole blood, 1 x 10^6 of cultured cells, or 200 µl of swab lysate. Purified RNA is suitable for all major downstream applications such as RNA-Seq, RT-PCR, NGS, and hybridization applications.

Process

The HighPrep™ Total RNA Plus kit uses a simple 4 steps procedure: Lyse+Bind-Wash-Elute. Tissue, blood, or cells are lysed, and released DNA and RNA binds to the HighPrep™ MAG-R4 magnetic beads in one step. Utilizing a magnetic separation device, the bound genomic DNA and RNA are separated from the solution and 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 Plus Kit Catalog No.	**HPTOR-R5**	**HPTOR-R50**	**HPTOR-R100**	**HPTOR-R100x4**	**STORAGE**
Number of Preps	5	50	100	400	
Solution A	5 mL	50 mL	100 mL	400 mL	15-25°C
LB Buffer	2.8 mL	28 mL	56 mL	224 ml	15-25°C
CE Buffer[1]	0.8 mL	8 mL	16 mL	64 mL	15-25°C
RW1 Buffer[1]	2 mL	20 mL	40 mL	160 mL	15-25°C
RB2 Buffer[1]	2 mL	20 mL	40 mL	160 mL (80mL x 2)	15-25°C
Pro K Solution	0.11 mL	1.1 mL	2.2 mL	8.8 mL	2-8°C
DNase I	0.011 mL	0.110 mL	0.22 mL	0.88 mL	-20°C
DNase I Digestion Buffer	0.6 mL	6 mL	12 mL	48 mL	15-25°C
RNA Elution Buffer	1 mL	10 mL	16 mL	64 mL	15-25°C
MAG-R4 Particles	0.055 mL	0.55 mL	1.1 mL	4.4 mL	2-8°C

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

Stability

All components are stable for 14 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% Ethanol	Storage
HPTOR-R5	**CE Buffer**	2 mL	Room Temp 15-25°C
	RW1 Buffer	1.25 mL	Room Temp 15-25°C
	RB2 Buffer	8 mL	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
HPTOR-R50	**CE Buffer**	20 mL	Room Temp 15-25°C
	RW1 Buffer	12.5 mL	Room Temp 15-25°C
	RB2 Buffer	80 mL	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
HPTOR-R100	**CE Buffer**	40 mL	Room Temp 15-25°C
	RW1 Buffer	25 mL	Room Temp 15-25°C
	RB2 Buffer	160 mL	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
HPTOR-R400	**CE Buffer**	160 mL	Room Temp 15-25°C
	RW1 Buffer	100 mL	Room Temp 15-25°C
	RB2 Buffer	320 mL per bottle	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

HighPrep™ Total RNA Plus - Tissue & Cultured Cells 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.

- ☐ Nuclease-free 1.5 ml microcentrifuge tubes
- ☐ Magnetic separation device
- ☐ Equipment for disrupting and homogenizing tissue
- ☐ 100% Ethanol
- ☐ Water bath, incubator, or heat block capable of 65°C

Things to do before starting

- ☐ Ensure the work area is RNase free
- ☐ Prepare reagents according to Preparation of Reagents Section
- ☐ Preset water bath, incubator or heating blocks to 65°C
- ☐ Completely resuspend the **MAG-R4 Particles** by vortexing

Protocol - 1.5 ml tube format

1. Homogenize the tissue or cells samples using an appropriate method. See below for examples of common homogenization methods:

   • For tissue samples

   1) Mortar and Pestle: Collect ~30mg fresh or preserved tissue sample in a mortar and freeze in liquid nitrogen. Grind the tissue using a clean pestle. Transfer the ground powder and liquid nitrogen into a 1.5 mL tube and allow the liquid nitrogen to evaporate. Add 500 µl of **LB Buffer** and vortex immediately and thoroughly.

   2) Bead-beating: Grind the ~30mg tissue in 500 µl of **LB Buffer** in a commercial bead-beater. Parameters such as grinding speed, duration, temperature, and type of beads, and etc. may need to be optimized. Refer to the manufacturer's manual for additional instructions.

   ⚠ ***Pro K Solution*** *is not necessary when using a clean mortar or bead-beating to grind tissue samples.*

   • For cultured cells

   Resuspend the $1x10^6$ cultured cell pellet in 500 µl of **LB Buffer** by vortexing or pipetting up-and-down thoroughly. Add 20 µl of **Pro K**, mix immediately by vortexing for 20 seconds, and incubate the sample at room temperature for 5 min. Vortex briefly once during incubation. If desired, using a rotor-stator homogenizer or passing through a 20-gauge needle may increase the yield.

2. Centrifuge the sample at 10,000 x g for 10 minutes at 4°C. Transfer the clear lysate to a new tube. Do not disturb the debris pellet.

3. Add **CE Buffer** to the lysate in a 1:1 ratio (i.e. for 400 µL of lysate add 400 µL of **CE Buffer**), and 10 µL of **MAG-R4 Particles** to each sample, vortex to mix thoroughly and incubate at room temperature for 10 minutes.

   ⚠ **CE Buffer** *must be diluted with ethanol prior to use. Complete resuspension of the magnetic particles is critical for obtaining high quality RNA.*

4. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

5. Add 600 µl of **RW1 Buffer** to the sample and re-suspend the magnetic particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.

   ⚠ ***RW1 Buffer*** *must be diluted with ethanol prior to use. Complete resuspension of the magnetic particles is critical for obtaining high quality RNA.*

6. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

7. Add 600 µl of **RB2 Buffer** to the sample and re-suspend the magnetic particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times**.**

   ⚠ *Complete resuspension of the magnetic particles is critical for obtaining high quality RNA.* ***RB2 Buffer*** *must be diluted with ethanol before use.*

8. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

   ⚠ *All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait for one minute and remove any residual liquid from the well using a fine pipet tip.*

9. Leave the tubes on the magnetic separation device for 5 minutes to air dry the **MAG-R4 Particles**.

10. While the samples are drying, prepare the DNase I mixture. For each sample, gently mix 98 µl of **DNase I Digestion Buffer** and 2 µl of **DNase I**.

11. Add 100 µl of the DNase I mixture to each sample. Gently mix by pipetting up and down to fully resuspend the magnetic beads. Incubate the samples at room temperature for 10 minutes.

    ⚠ *Avoid extensive vortexing or pipetting as this may denature the* ***DNase I.***

12. Add 600 µl of **RB2 Buffer** to the sample and resuspend the magnetic particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times. Incubate the samples at room temperature for 1 minute.

    ⚠ **RB2 Buffer** *must be diluted with ethanol before use.*

13. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

14. Repeat Steps 12-13 for a second RNA wash.

15. Leave the tubes on the magnetic separation device for 5-10 minutes to air dry the M**AG-R4 Particles.** Remove any residual liquid with a fine pipet tip.

    ⚠ *It is critical to completely remove all liquid from each tube.*

16. Add 50-100 µl of **RNA Elution Buffer**. Completely resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.

17. Incubate for 10 minutes at room temperature.

    ⚠ *Incubation at 65°C may improve the yield for some tissue samples, but it may decrease the sample's RNA integrity number (RIN) values.*

18. Place the tubes back on the magnetic separation device and wait 2-5 minutes or until the magnetic particles are completely cleared from the **RNA Elution Buffer.**

19. Transfer the cleared supernatant containing the purified RNA to a new 1.5 mL tube.

20. Store the RNA at –80°C.

HighPrep™ Total RNA Plus - Whole Blood 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.

- ☐ Nuclease-free 1.5 ml microcentrifuge tubes
- ☐ Magnetic separation device
- ☐ 100% Ethanol
- ☐ Water bath, incubator, or heat block capable of 65°C

Things to do before starting

- ☐ Ensure the work area is RNase free
- ☐ Prepare reagents according to Preparation of Reagents Section
- ☐ Preset water bath, incubator or heating blocks to 65°C
- ☐ Completely resuspend the **MAG-R4 Particles** by vortexing

Protocol - 1.5 ml tube format

1. Add 200 µl of whole blood in a 1.5 ml microcentrifuge tube and 600 µl of **Solution A**.

2. Mix well and centrifuge at 10,000 x g for 5 min at 4°C. Decant the supernatant and keep the pellet.

3. Add 200 µl of **Solution A** and mix gently to break the pellet.

4. Centrifuge the sample at 10,000 x g for 5 min at 4°C for the final wash. Decant the supernatant and keep the pellet.

5. Add 500 µl of **LB Buffer** to the pellet and 20 µl of **Pro K**, incubate the sample at room temperature for 20 min. Vortex briefly once during incubation.

6. Centrifuge the sample at 10,000 x g for 10 minutes at 4°C. Transfer the clear lysate to a new tube. Do not disturb the debris pellet.

7. Add **CE Buffer** to the lysate in a 1:1 ratio (i.e. for 400 µL of lysate add 400 µL of **CE Buffer**), and 10 µL of **MAG-R4 Particles** to each sample. Vortex to mix thoroughly and incubate at room temperature for 10 minutes.

   ⚠ **CE Buffer** *must be diluted with ethanol prior to use. Complete resuspension of the magnetic particles is critical for obtaining high quality RNA.*

8. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

9. Add 600 µl of **RW1 Buffer** to the sample and re-suspend the magnetic particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.

   ⚠ **RW1 Buffer** *must be diluted with ethanol prior to use. Complete resuspension of the magnetic particles is critical for obtaining high quality RNA.*

10. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

11. Add 600 µl of **RB2 Buffer** to the sample and re-suspend the magnetic particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.

    ⚠ *Complete resuspension of the magnetic particles is critical for obtaining high quality RNA.* **RB2 Buffer** *must be diluted with ethanol before use.*

12. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

    ⚠ *All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait for one minute and remove any residual liquid from the well using a fine pipet tip.*

13. Leave the tubes on the magnetic separation device for 5 minutes to air dry the **MAG-R4 Particles.**

14. While the samples are drying, prepare the DNase I mixture. For each sample, gently mix 98 µl of **DNase I Digestion Buffer** and 2 µl of **DNase I**.

    ⚠ *Avoid extensive vortexing or pipetting as this may denature the* ***DNase I****.*

15. Add 100 µl of DNase I mixture to each sample. Gently mix by pipetting up and down to fully resuspend the magnetic beads. Incubate the samples at room temperature for 10 minutes.

16. Add 600 µl of **RB2 Buffer** to the sample and resuspend the magnetic particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times. Incubate the samples at room temperature for 1 minute**.**

    ⚠ ***RB2 Buffer*** *must be diluted with ethanol before use.*

17. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

18. Repeat Steps 16-17 for a second RNA wash.

19. Leave the tubes on the magnetic separation device for 5-10 minutes to air dry the **MAG-R4 Particles**. Remove any residual liquid with a fine pipet tip**.**

    ⚠ *It is critical to completely remove all liquid from each tube.*

20. Add 50-100 µl of **RNA Elution Buffer**. Completely resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.

21. Incubate for 10 minutes at room temperature.

    ⚠ *Incubation at 65°C may improve the yield for some tissue samples, but it may decrease the sample's RNA integrity number (RIN) values.*

22. Place the tubes back on the magnetic separation device and wait 2-5 minutes or until the magnetic particles are completely cleared from the **RNA Elution Buffer**.

23. Transfer the cleared supernatant containing the purified RNA to a new 1.5 mL tube.

24. Store the RNA at –80°C.

HighPrep™ Total RNA Plus - Buccal Cells 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.

- ☐ Nuclease-free 1.5 ml microcentrifuge tubes
- ☐ Magnetic separation device
- ☐ 100% Ethanol
- ☐ Water bath, incubator, or heat block capable of 65°C

Things to do before starting

- ☐ Ensure the work area is RNase free
- ☐ Prepare reagents according to Preparation of Reagents Section
- ☐ Preset water bath, incubator or heating blocks to 65°C
- ☐ Completely resuspend the **MAG-R4 Particles** by vortexing

Protocol - 1.5 ml tube format

1. Add 500 µL of **LB Buffer** to 200 µL of the swab, mix by vortexing or pipetting up and down thoroughly. Incubate the sample at room temperature for 5 min. Vortex briefly once during Incubation. If desired, using a rotor-stator homogenizer or passing through 20-gauge needle may increase yield.

2. Centrifuge the sample at 10,000 x g for 10 minutes. Transfer the clear lysate to a new tube. Do not disturb the debris pellet.

3. Add **CE Buffer** to the lysate in a 1:1 ratio (i.e. for 400 µL of lysate add 400 µL of **CE Buffer**), and 10 µL of **MAG-R4 Particles** to each sample. Vortex to mix thoroughly and incubate at room temperature for 10 minutes.

   ⚠ **CE Buffer** *must be diluted with ethanol prior to use. Complete resuspension of the magnetic particles is critical for obtaining high quality RNA.*

4. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

5. Add 600 µL of **RW1 Buffer** to the sample and re-suspend the magnetic particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.

   ⚠ ***RW1 Buffer*** *must be diluted with ethanol prior to use. Complete resuspension of the magnetic particles is critical for obtaining high quality RNA*

6. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

7. Add 600 µL of **RB2 buffer** to the sample and re-suspend the magnetic particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.

   ⚠ *Complete resuspension of the magnetic particles is critical. **RB2 Buffer** must be diluted with ethanol before use.*

8. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

   ⚠ *All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well using a fine pipet tip.*

9. Leave the tube on the magnetic separation device for 5 minutes to air dry the **MAG-R4 Particles**.

10. While the samples are drying, prepare the DNase I mixture. For each sample, gently mix 98 µL of **DNase I Digestion Buffer** and 2 µL of **DNase I**.

11. Add 100 µL DNase I mix to each sample. Mix by pipetting up and down to fully re-suspend the magnetic beads. Incubate the samples at room temperature for 10 minutes.

    ⚠ *Avoid extensive vortexing or pipetting as this may denature the **DNase I**.*

12. Add 600 µL of **RB2 Buffer** to the sample and resuspend the magnetic particles by vortexing at maximum speed for 1 min or by pipetting up and down 10 times. Incubate the samples at room temperature for 1 minute.

    ⚠ ***RB2 Buffer** must be diluted with ethanol before use.*

13. Place the sample tubes on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

14. Repeat Steps 12-13 for a second RNA wash.

15. Leave the tube on the magnetic separation device for 5-10 minutes to air dry the **MAG-R4 Particles**. Remove any residual liquid with a fine pipet tip.

    ⚠ *It is critical to completely remove all liquid from each tube.*

16. Add 30-50 µL of **RNA Elution Buffer**. Completely resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 min or by pipetting up and down 10 times.

    ⚠ *For concentrated RNA use low elution volume.*

17. Incubate for 10 minutes at room temperature.

    ⚠ *Incubation at 65°C may improve the yield.*

18. Place the tubes back on the magnetic separation device and wait 2-5 minutes or until the magnetic particles are completely cleared from the **RNA Elution Buffer.**

19. Transfer the cleared supernatant containing purified RNA to a new 1.5 ml tube, and store purified RNA at -80 ℃.

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 RNA yield	Incomplete resuspension of MAG-R4 Particles	Resuspend the MAG-R4 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 cell count will give reduced yield.
	Incomplete resuspension of MAG-R4 Particles	Resuspend MAG-R4 Particles by vortexing vigorously before use.
	Loss of MAG-R4 Particles during procedure	Be careful not to remove the MAG-R4 Particles during the procedure.
	Ethanol was not added to Wash Buffers	Add ethanol to Wash Buffers as instructed on Page 4.
	MAG-R4 Particles not resuspended during binding	Vortex vigorously for 2 minutes after addition of ethanol and MAG-R4 Particles.
Problem with downstream application	Insufficient RNA was used	RNA in the sample already degraded. Do not freeze/thaw the sample more than once. Do not store at room temperature.
	Ethanol carry-over	Dry the MAG-R4 Particles completely before elution.
Carryover of the magnetic particles in the elution	Carryover of the MAG-R4 Particles in the eluted RNA will not affect downstream applications	To remove the carryover MAG-R4 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 Plus - 50 preps	HPTOR-R50	50
HighPrep™ Total RNA Plus - 100 preps	HPTOR-R100	100
HighPrep™ Total RNA Plus - 400 preps	HPTOR-R400	400

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. All biological samples are considered potentially infectious. When working with the samples and chemicals always wear a suitable lab coat, disposable gloves, and protective goggles. For more information please consult the appropriate Material Safety Data Sheets (MSDSs).


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