MAGBIO
"WE MAKE NGS BETTER"

HighPrep Total RNA Plus Kit

Catalog Nos. HPTOR-R5, HPTOR-R50, HPTOR-R100, HPTOR-R400

Protocol for Total RNA Extraction from Buccal Cells Collected and Stabilized with iSWAB-RNA v2

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

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			

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1. Collected buccal cells will settle at the bottom of the iSWAB-RNA-v2 tube. Tip mix gently 3-5 times with a pipette using RNAse/DNase free 200 µL tip or rock the iSWAB-RNA-v2 tube for 30 minutes to mix the sample. Then transfer **600 µL** from the iSWAB-RNA-v2 tube to a DNase/RNase free Eppendorf tube.

2. Centrifuge the cells at 5000 x *g* for 10 mins and remove the supernatant.

3. Add 500 µL of LB Buffer to the cells, mix by pipetting up-and-down thoroughly. Incubate the sample at room temperature for 5 min. Mix briefly once during incubation.

4. 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.

5. Add CE buffer to the lysate in 1:1 ratio (i.e. for 500 µL of lysate add 500 µL of CE buffer), and 10 µL MAG-R4 Particles to each sample, pipette mix thoroughly and incubate at room temperature for 10 minutes.

   ***Note**: CE 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 RW1 Buffer to the sample and re-suspend the magnetic particles by by pipetting up and down 10 times.

   ***Note**: RW1 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 RB2 buffer to the sample and re-suspend the magnetic particles by pipetting up and down 10 times.

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

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 solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

    Note: 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.

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

12. 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.

13. 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.

    Note: Avoid extensive vortexing or pipetting as this may denature the DNase I.

14. Add 600 µL of RB2 Buffer to the sample and resuspend the magnetic particles by pipetting up and down 10 times. Incubate the samples at room temperature for 1 minute.

    ***Note**: RB2 Buffer must be diluted with ethanol before use.*

15. 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.

16. Repeat Steps 14-15 for a second and third RNA wash.

17. 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.

    ***Note**: it is critical to completely remove all liquid from each tube.*

18. Add 30-50 µL of RNA Elution Buffer. Completely resuspend the MAG-R4 Particles by pipetting up and down 10 times.

19. Incubate for 10 minutes at 37°C.

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

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

Troubleshooting Guide

Symptoms	Possible Causes	Comments
Low RNA Yield	RNA degraded during storage	Make sure the samples are properly stored. Do not freeze iSWAB-RNA v2 samples immediately after collection, samples should be kept at room temperature for at least 3 days before processing. However, the samples are good for 10 days at room temperature. After 10 days at room temperature samples can then be frozen at -80°C until processed.
	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 CE buffer or the Wash Buffers	Add ethanol to Wash Buffers as instructed on Page 4.
	MAG-R4 Particles not resuspended during binding	Mix the sample and MAG-R4 Particles very well after addition of CE buffer and MAG-R4 Particles.
Problem with downstream application	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.
No bands on the Tapestation gel	Tapestation sample buffer and sample elution buffer incompatibility	Some sample elution buffers when used for iSWAB-RNA v2 sample elution will not run well on the Tapestation and sometimes even the lower marker will not show on the gel. The best way to elute RNA from iSWAB-RNA v2 samples is by using RNase free water.
RNA appears degraded on the gel	RNA degraded during processing	Follow the protocol as it is without modifications; do not use liquid nitrogen, it will affect the quality of buccal cells collected with iSWAB-RNA v2 as well as using a needle to disrupt cells.

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Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs).

TRADEMARKS

Ordering Information

Product Description	Catalog No.	Preps
HighPrep™ Total RNA Plus kit - 50 preps	HPTOR-R50	50
HighPrep™ Total RNA Plus kit - 100 preps	HPTOR-R100	100
HighPrep™ Total RNA Plus kit - 400 preps	HPTOR-R400	400