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HighPrep™ Total RNA Isolation Kit - iSWAB™

Protocol for Total RNA Extraction from Buccal Cells Collected and Stabilized in iSWAB-RNA v2 Collection Kit

Manual Revision v1.00
Catalog Nos. ISTOR-R5, ISTOR-R50, ISTOR-R100, ISTOR-R100X4

- RNA isolation from buccal cells
- Magnetic beads 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 Isolation Kit - iSWAB™ is specially designed for purifying total RNA from buccal cells collected and stabilized in iSWAB-RNA v2 tube (Mawi DNA Technologies). The kit uses the special lysis condition with HighPrep™ magnetic particles technology to isolate high-quality total RNA from 600 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 Isolation Kit - iSWAB™ uses a simple 4 steps procedure: Lyse+Bind-Wash-Elute. Buccal 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 bead washes to remove any contaminants, RNA is eluted from magnetic beads.

Kit Contents and Storage

HighPrep™ Total RNA Isolation Kit - iSWAB™ Catalog No.	**ISTOR-R5**	**ISTOR-R50**	**ISTOR-R100**	**ISTOR-R100x4**	**STORAGE**
Number of Preps	5	50	100	400	
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
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
ISTOR-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
ISTOR-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
ISTOR-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
TOR-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 Isolation Kit - iSWAB™
Buccal Cells Collected and Stabilized in iSWAB-RNA v2 Collection Kit

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 37°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 37°C
- ☐ Remove the **MAG-R4 Particles** from the fridge to thaw 30 minutes prior to performing the experiment.

Protocol

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. Using the 200 µL tip, transfer 600 µL of the sample from the iSWAB-RNA-v2 tube to a DNase/RNase free Eppendorf tube.

   ⚠ *The 1000 µL tip cannot fit into the iSWAB-RNA-v2 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.

   ⚠ *The MAG-R4 Particles must be vortexed prior to use.*
   ⚠ *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 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.*

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.

   ⚠ *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.

    ⚠ *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 of 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.

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.

    ⚠ *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 the 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.

    ⚠ *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

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	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.

Ordering Information

Product Description	Catalog No.	Preps
HighPrep™ Total RNA Isolation Kit- iSWAB™ - 50 preps	ISTOR-R50	50
HighPrep™ Total RNA Isolation Kit- iSWAB™ - 100 preps	ISTOR-R100	100
HighPrep™ Total RNA Isolation Kit- iSWAB™ - 400 preps	ISTOR-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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