MAGBIO

"WE MAKE NGS BETTER"

HighPrep RNA Elite - DX

Purification of RNA or cDNA for *in vitro* applications as well as RNA and cDNA probe synthesis

Catalog Nos. RC-90001E, RC-90005E, RC-90050E, RC-90250E, RC-90500E
Manual Revision 1
WI-72-64

- Magnetic beads based chemistry
- No centrifugation or filtration

Instructions For Use

Contents

EC REP **CEpartner4U**
Esdoornlaan 13, 3951 DB Maarn
Netherlands
www.cepartner4u.com

UK REP **UKCApartner4U**
7 Campion Way, Bingham, Nottingham, NG13 8TR
United Kingdom
www.ukcapartner4u.co.uk

CE IVD MHRA

For *in vitro* diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

HighPrep RNA Elite - DX utilizes MagBio's solid-phase paramagnetic bead technology for high-throughput purification of RNA or cDNA for *in vitro* applications such as transcription, antisense RNA (aRNA) amplification as well as RNA and cDNA probe synthesis. This protocol enables purification of micro RNA (miRNA), small RNA, and total RNA from enzymatic reactions, concentrating miRNA and total RNA from a diluted sample. This protocol can be used for manual procedure as well as a guideline for adapting the kit to automated instruments. For availability of ready-to-run scripts using the KingFisher™ Flex, please contact MagBio Genomics.

Products purified with HighPrep RNA Elite - DX are ready to be used in the following applications:

- PCR and RT-PCR reactions
- Transfection for RNAi experiments
- Probes for microarray or macroarray
- cDNA synthesis and labeling
- *In* vitro RNA synthesis
- Next generation sequencing

Process

HighPrep RNA Elite - DX uses a simple 3 steps procedure: Bind-Wash-Elute. HighPrep RNA Elite - DX is added to the reaction sample. The protocol utilizes a magnet plate or magnet stand for magnetization of the beads during sample processing. During the process, contaminants and salts are washed off and pure RNA is eluted, ready to be used in subsequent applications.

Product Specifications

Catalog Number	Description	Number of Reactions	Storage Conditions
RC-90001E	HighPrep RNA Elite - DX (1 mL)	55	2-8°C DO NOT FREEZE
RC-90005E	HighPrep RNA Elite - DX (5 mL)	277	
RC-90050E	HighPrep RNA Elite - DX (50 mL)	2,777	
RC-90250E	HighPrep RNA Elite - DX (250 mL)	13,888	
RC-90500E	HighPrep RNA Elite - DX (500 mL)	27,777	

Number of reactions is based on typical 10 µL reaction volume.
Volume of HighPrep RNA Elite - DX per reaction = 1.8 x (Reaction Volume)

Storage and Preparation

- Store at 2-8°C. **DO NOT FREEZE.**
- Keep at room temperature for 30 minutes prior to use.
- Thoroughly shake the HighPrep RNA Elite - DX to resuspend the beads before use.

Materials Supplied in the Kit

- HighPrep RNA Elite - DX

Equipment and Reagents to Be Supplied by the User:

- 80% Ethanol (Use freshly prepared 80% Ethanol)
- 70% Ethanol or RNase inhibiting surfactant solution
- RNase-free water, TRIS-HCl pH 8.0, or TE Buffer
- Hydrogen Peroxide (3%)
- DEPC treated water
- Magnetic separation device compatible with 96 or 384 well PCR plate (see page 5)
- 96-well PCR plate or 384-well PCR plate
- Multichannel pipette
- Sterile aerosol barrier (filtered) pipette tips (DNase and RNase Free)
- Polypropylene reservoirs
- Cold block or ice
- Laboratory mixer, vortex, or equivalent

Working In RNase Free Conditions

RNases are present everywhere and some general precautions should be followed in order to avoid the introduction of contaminating nucleases during the HighPrep RNA Elite - DX procedure. The most common sources of RNase contamination are hands, dust particles, and contaminated laboratory instruments, solutions and glassware. The following procedures should be followed to limit RNase contamination when working with RNA:

- Always wear gloves while working and change gloves frequently.
- Refrain from using reagents, consumables, and equipment that are in common use for other general lab processes.
- Use dedicated RNase free equipment such as pipettes, pipette tips, gels boxes, etc.
- Work in a separate room (fume hood or lab space if available).
- Use plastic, disposable consumables that are certified RNase free.
- Purchase reagents, such as commonly used buffers and water, that are certified RNase free. Prepare small individual aliquots of such buffers to avoid repeated transfer of out of stock buffers. This lowers the risk of contamination of the stock solution.
- Wipe down work surfaces with commercial RNase inhibiting surfactant solutions or 70% Ethanol before starting work.

Before use, treat electrophoresis gel boxes, including combs and gel trays, with 3% hydrogen peroxide for 10 minutes and rinse with DEPC treated water.

HighPrep RNA Elite - DX: RNA cleanup reaction using a 96-well plate

⚠ Bring the **HighPrep RNA Elite - DX** to room temperature for at least 30 min before use.

1. Shake thoroughly the **HighPrep RNA Elite - DX** to fully resuspend the magnetic beads.

2. Transfer RNA sample to an appropriate 96-well plate. Follow the table in step 3 for the desired sample processing volume.

3. Add **HighPrep RNA Elite - DX** volume according to the RNA sample.
   See table below to determine appropriate volume.

cDNA or RNA Reaction Volume (µL)	HighPrep RNA Elite - DX Volume at 1.8X (uL)*
10	18
20	36
50	90

* Formula used to calculate the volume of HighPrep RNA Elite - DX needed for RNA reaction:
HighPrep RNA Elite - DX volume per reaction = 1.8 X cDNA or RNA reaction volume.

4. Mix the **HighPrep RNA Elite - DX** and sample by pipetting up and down 6-8 times.

5. Incubate the mixture for 5 minutes at room temperature.

6. Place the sample plate on the 96 magnetic separation device for 3 minutes or until the solution clears. Beads will pull to the side of the well.

7. With the sample plate still on the magnet, remove and discard the supernatant by pipetting.

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

8. With the sample plate on the magnet, add 200 µL of 80% Ethanol to each well and incubate for 30 seconds at room temperature.

9. With the plate still on the magnet, remove and discard the supernatant by pipetting.

10. Repeat steps 8 - 9 for a total of two 80% Ethanol washes.

11. Dry the beads by incubating the plate for 10 minutes at room temperature with the plate still on the magnetic separation device.

    ⚠ *It is critical to completely remove all traces of alcohol but take caution in not over drying the beads as this will reduce the yield.*

12. Remove the sample plate from the magnetic separation device. Add 40 µL of elution buffer (RNase-free water, TRIS-HCl pH 8.0, or TE buffer) to each well and pipette up and down 10 times to mix. Prewarming the elution buffer to 55°C can increase the yield.

13. Incubate for 2 minutes at room temperature.

14. Place the sample plate back on the magnetic separation device and wait 3 minutes or until the magnetic beads clear from the solution.

15. Transfer the eluate (cleared supernatant) to a new plate for subsequent applications or store at -80°C for later use.

HighPrep RNA Elite - DX: RNA cleanup reaction using a 384-well plate

⚠ Bring the **HighPrep RNA Elite - DX** to room temperature for at least 30 min before use.

1. Shake thoroughly the **HighPrep RNA Elite - DX** to fully resuspend the magnetic beads.

2. Transfer RNA sample to an appropriate 384-well plate. Follow the table in step 3 for the desired sample processing volume.

3. Add **HighPrep RNA Elite - DX** volume according to the RNA sample.
   See table below to determine appropriate volume.

cDNA or RNA Reaction Volume (µL)	HighPrep RNA Elite - DX Volume at 1.8X (uL)*
5	9
7	12.6
10	18

* Formula used to calculate the volume of HighPrep RNA Elite - DX needed for RNA reaction:
HighPrep RNA Elite - DX volume per reaction = 1.8 X cDNA or RNA reaction volume.

4. Mix the **HighPrep RNA Elite - DX** and sample by pipetting up and down 6-8 times.

5. Incubate the mixture for 5 minutes at room temperature.

6. Place the sample plate on the 384 magnetic separation device for 2 minutes or until the solution clears. Beads will pull to the side of the well.

7. With the sample plate still on the magnet, remove and discard the supernatant by pipetting.

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

8. With the sample plate on the magnet, add 30 µL of 80% Ethanol to each well and incubate for 30 seconds at room temperature.

9. With the plate still on the magnet, remove and discard the supernatant by pipetting.

10. Repeat steps 8 - 9 for a total of two 80% Ethanol washes.

11. Dry the beads by incubating the plate for 3-5 minutes at room temperature with the plate still on the magnetic separation device.

    ⚠ *It is critical to completely remove all traces of alcohol but take caution in not over drying the beads as this will reduce the yield.*

12. Remove the sample plate from the magnetic separation device. Add 30 µL of elution buffer (RNase-free water, TRIS-HCl pH 8.0, or TE buffer) to each well and pipette up and down 10 times to mix. Prewarming the elution buffer to 55°C can increase the yield.

13. Incubate for 2 minutes at room temperature.

14. Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from the solution.

15. Transfer the eluate (cleared supernatant) to a new plate for subsequent applications or store at -80°C for later use.

Ordering Information

HighPrep RNA Elite - DX

Catalog No.	Product
RC-90005E	HighPrep RNA Elite - DX (5 mL)
RC-90050E	HighPrep RNA Elite - DX (50 mL)
RC-90250E	HighPrep RNA Elite - DX (250 mL)
RC-90500E	HighPrep RNA Elite - DX (500 mL)

Related Products

Total RNA Isolation

Catalog No.	Product	Preps
HPTOR-R50E	HighPrep Total RNA Plus Kit - DX (50 preps)	50
HPTOR-R100E	HighPrep Total RNA Plus Kit - DX (100 preps)	100
HPTOR-R100x4E	HighPrep Total RNA Plus Kit - DX (400 preps)	400

Magnetic Separation Devices

Catalog No.	Description
MYMAG-96	Handheld Magnetic Separation Device (96 well microplate format)
MYMAG-96X	Magnetic Separation Device (96 well ring format)
MBMS-12	MagStrip Magnet Stand (1.5 mL x 12)
MBMS-31550	15 mL and 50 mL Magnetic Stand Combo (3 x 15 mL and 3 x 50 mL)

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