



"WE MAKE NGS BETTER"

HighPrep™ RNA Elite

Catalog Nos. RC-90005, RC-90050, RC-90250, RC-90500
Manual Revision v3

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

- Magnetic beads based chemistry
- No centrifugation or filtration

PROTOCOL

Contents

Product Description and Process.....	1
Product Specifications.....	1
96 well protocol	3
384 well protocol	4

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

Information in this document is subject to change without notice.

MAGBIO GENOMICS, INC. DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL MAGBIO GENOMICS, INC. BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT MAGBIO GENOMICS, INC. IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

TRADEMARKS

The trademarks mentioned herein are the property of MagBio Genomics, Inc. or their respective owners.

Product Description

The HighPrep™ RNA Elite system 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 recovery 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 guideline for adapting the kit to automated liquid handling instruments.

Product purified with the HighPrep RNA Elite system 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

Process

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

Product Specifications

Product Number	Description	Number of Reactions	Storage Conditions
RC-90005	HighPrep™ RNA Elite- 5 mL	278	2-8°C DO NOT FREEZE
RC-90050	HighPrep™ RNA Elite - 50 mL	2,778	
RC-90250	HighPrep™ RNA Elite- 250 mL	13,890	
RC-90500	HighPrep™ RNA Elite - 500 mL	27,780	

Number of reactions is based on typical 10µL reaction volume.

Volume of HighPrep™ RNA Elite reagent per reaction = 1.8 x (Reaction Volume)

Materials Supplied in the Kit

- HighPrep™ RNA Elite paramagnetic beads solution
- Store at 2-8°C. DO NOT FREEZE. HighPrep™ RNA Elite is stable for 14 months when stored at 2-8°C.
- Thoroughly shake the HighPrep™ RNA Elite reagent to resuspend the beads before use.

Equipment and Reagents to Be Supplied by User:

- 80% ethanol, RNase free (Use Freshly prepared 80% ethanol)
- Reagent grade water, RNase free

Magnet (Stand and Plate):

For 1.5mL tube format: MagBio MagStand12 - Magnet Stand (1.5ml x 12)

MagBio Genomics, Inc., Cat# MBMS-12, www.magbiogenomics.com

For 96 well format: 96 well ring plate

MagBio Genomics, Inc., Cat# MYMAG-96X, www.magbiogenomics.com

For 384 well format: 384 magnet plate

Reaction Plate:

For 96 well format: 96 well cycling plate

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 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 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 - 96 Well Format

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

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

2. **Transfer RNA reaction to appropriate 96-well plate.**

For 50 µl reaction, adjust volume using sterile water.

3. **Add HighPrep RNA™ Elite reagent volume according to the RNA reaction.**

See table below to determine appropriate volume.

Reaction Volume (µL)	HighPrep RNA Elite Volume at 1.8X (uL)*
10	18
14	25

* Formula used to calculate the volume of HighPrep™ RNA Elite reagent needed for RNA reaction:

HighPrep™ RNA Elite reagent volume per reaction = 1.8 X PCR reaction volume.

4. **Mix thoroughly the HighPrep™ RNA Elite reagent 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 15 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 three 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 (reagent grade 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 storage or for subsequent applications.**

HighPrep™ RNA Elite - 384 Well Format

- Shake thoroughly the HighPrep RNA Elite reagent to fully resuspend the magnetic beads.**
* Bring the HighPrep RNA Elite to room temperature for at least 30 min before use.
- Transfer RNA reaction to appropriate 384-well plate.**
For 50 µl reaction, adjust volume using sterile water.

- Add HighPrep™ RNA Elite reagent volume according to the PCR reaction.**
See table below to determine appropriate volume.

Reaction Volume (µL)	HighPrep RNA Elite Volume at 1.8X (µL)*
5	9
7	12.6

* Formula used to calculate the volume of HighPrep RNA Elite reagent needed for reaction:
HighPrep™ RNA Elite reagent volume per reaction = 1.8 X reaction volume.

- Mix thoroughly the HighPrep™ RNA Elite reagent and sample by pipetting up and down 6-8 times.**
- Incubate the mixture for 5 minutes at room temperature.**
- 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.
- 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.
- With the sample plate on the magnet, add 30 µl of 80% ethanol to each well and incubate for 30 seconds at room temperature.**
- With the plate still on the magnet, remove and discard the supernatant by pipetting.**
- Repeat steps 8 - 9 for a total of two 80% ethanol washes.**
- 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.
- Remove the sample plate from the magnetic separation device. Add 30 µl of elution buffer (reagent grade 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.
- Incubate for 2 minutes at room temperature.**
- Place the sample plate back on the magnetic separation device and wait 2 minutes or until the magnetic beads clear from the solution.**
- Transfer the eluate (cleared supernatant) to a new plate for storage or for subsequent applications.**

Ordering and Related Product Information

Post PCR and Next Gen library prep clean up system

Catalog No.	Product
AC-60005	HighPrep PCR (5 mL)
AC-60050	HighPrep PCR (50 mL)
AC-60250	HighPrep PCR (250 mL)
AC-60500	HighPrep PCR (500 mL)

BigDye Sanger Sequencing Cleanup

Catalog No.	Product
DT-70005	HighPrep DTR (5 mL)
DT-70050	HighPrep DTR (50 mL)
DT-70250	HighPrep DTR (250 mL)
DT-70500	HighPrep DTR (500 mL)

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 magnetic stand (1.5 mL x 12)
MBMS-31550	15ml and 50ml magnetic stand combo. (3x15ml and 3x50ml)

cfDNA Purification Kit

Catalog No.	Product	Description	Preps
CFK-DI10-400UL	CF-Kapture Kit (200-400µl) 10 preps	Purification of cell-free DNA (cfDNA) from 200-400 µl STABILIZED plasma	10
CFK-DI5-5ML	CF-Kapture Kit (3-5ml) 5 preps	Purification of cell-free DNA (cfDNA) from 3-5 ml STABILIZED plasma	5
CFK-DI50-400UL	CF-Kapture Kit (200-400µl)(50 preps)	Purification of cell-free DNA (cfDNA) from 200-400 µl STABILIZED plasma	50
CFK-DI50-2ML	CF-Kapture Kit (1-2ml) 50 preps	Purification of cell-free DNA (cfDNA) from 1-2 ml STABILIZED plasma	50
CFK-DI50-5ML	CF-Kapture Kit (3-5 ml) 50 preps	Purification of cell-free DNA (cfDNA) from 3-5 ml STABILIZED plasma	50



www.magbiogenomics.com