MAGBIO

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HighPrep™ RNA Elite

Catalog Nos. RC-90005, RC-90050, RC-90250, RC-90500

Manual Revision v1.01

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

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

Information in this document is subject to change without notice.

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 automatic 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 (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	4-8°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 4°C. DO NOT FREEZE. HighPrep RNA Elite is stable for 14 months when stored at 4°C.
- Thoroughly shake the HighPrep™ RNA Elite reagent to resuspend the beads before use.

Equipment and Reagents to Be Supplied by User:

- 70% ethanol, RNase free (Use Freshly prepared 70% ethanol)
- Reagent grade water, RNase free

Magnet (Stand and Plate):

For 1.5mL tube format: MagBio MagStand10 - Magnet Stand (1.5ml x 10)
MagBio Genomics, Inc., Cat# MBMS-10, www.magbiogenomics.com
For 96 well format: 96 well ring plate
For 384 well format: 384 magnet plate

Reaction Plate:

For 96 well format: 96 well cycling plate
For 384 well format: 384 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 temperatue for at least 30 min before use.
2. **Transfer RNA reaction to appropriate 96-well plate.**
   For 50µl 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:
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 70% 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 70% 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 40µl of elution buffer (reagent grade water, TRIS-HCl pH 8.0 or TE buffer) to each well and pipet up and down 10 times to mix.** Prewarming the elution buffer at 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 minute or until the magnetic beads clear from solution.**
15. **Transfer the eluate (cleared supernatant) to a new plate for storage or for subsequent applications.**

HighPrep™ RNA Elite - 384 Well Format

1. **Shake thoroughly the HighPrep RNA Elite reagent to fully resuspend the magnetic beads.**
   * Bring the HighPrep RNA Elite to room temperatue for at least 30 min before use.
2. **Transfer RNA reaction to appropriate 384-well plate.**
   For 50µl reaction, adjust volume using sterile water.
3. **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 (uL)*
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.
4. **Mix thoroughly the HighPrep RNA Elite reagent and sample by mix 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 minute 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 70% 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 70% 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 30µl of elution buffer (reagent grade water, TRIS-HCl pH 8.0 or TE buffer) to each well and pipet up and down 10 times to mix.** Prewarming the elution buffer at 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 minute or until the magnetic beads clear from solution.**
15. **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-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-70500	HighPrep DTR (500 mL)

Magnetic Separation Devices

Catalog No.	Description
MYMAG-96	Handheld Magnetic Separation Device (96 well microplate format)
MBMS-10	MagStip magnetic stand (1.5 mL x 10)
MBMS-31550	15ml and 50ml magnetic stand combo. (3x15ml and 3x50ml)

cfDNA Purification Kit

Catalog No.	Product	Description	Preps
CFK-D10-400UL	CF-Kapture 21 Kit (200-400µl) 10 preps	Purification of cell-free DNA (cfDNA) from 200-400 µl STABILIZED plasma	10
CFK-D5-5ML	CF-Kapture 21 Kit (3-5ml) 5 preps	Purification of cell-free DNA (cfDNA) from 3-5 ml STABILIZED plasma	5
CFK-D50-400UL	CF-Kapture 21 Kit (200-400µl)(50 preps)	Purification of cell-free DNA (cfDNA) from 200-400 µl STABILIZED plasma	50
CFK-D50-2ML	CF-Kapture 21 Kit (1-2ml) 50 preps	Purification of cell-free DNA (cfDNA) from 1-2 ml STABILIZED plasma	50
CFK-D50-5ML	CF-Kapture 21 Kit (3-5 ml) 50 preps	Purification of cell-free DNA (cfDNA) from 3-5 ml STABILIZED plasma	50

Whole blood stabilization tubes

Catalog No.	Product	Description
BS21-CF10-100	Blood STASIS 21-ccfDNA 9 mL (100)	100 tubes: 2 ml Additive, 7 ml blood draw volume
BS21-CF6-100	Blood STASIS 21-ccfDNA 6 mL (100)	100 tubes: 1.5 ml Additive, 4.5 ml blood draw volume
BS21-CF3-200	Blood STASIS 21-ccfDNA 3 mL (200)	200 tubes: 0.5 ml Additive, 2.5 ml blood draw volume

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