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NextPrep™ Magnazol™ cfRNA Isolation Kit

(Magnetic Bead-based Isolation of Cell-free RNA from Plasma & Serum)

Catalog #NOVA-3830-01 (Kit contains up to 25 isolations)

 V19.03

This product is for research use only.
Not for use in diagnostic procedures.

NextPrep™ Magnazol™ cfRNA Isolation Kit - NOVA-3830-01

Product Overview

The NextPrep™ Magnazol™ cfRNA Isolation Kit is designed for extracting circulating RNA from the cell-free fraction of blood, i.e. plasma and serum, using a magnetic bead format. The Magnazol™ Extraction Reagent is a single-phase reagent containing guanidinium, a powerful chaotropic agent effective for rapidly inactivating nucleases, and phenol, an organic solvent used to denature and separate proteins and DNA from RNA. The reagent is effective for recovering cfRNA associated with proteins and with membrane-bound particles such as platelets and exosomes.

Features of the Product:

- Recovers high yields of total RNA, including small RNA, from human plasma and serum
- Allows high volume of sample (up to 0.6 mL) to be processed in single microcentrifuge tube format
- Includes BCP (alternative to chloroform) for phase separation
- Uses magnetic beads for rapid purification of RNA from aqueous phase
- Validated for isolation of RNA for producing Small RNA-Seq libraries

Plasma or serum is mixed with 2 volumes of the Magnazol™ Extraction Reagent and then with 0.2 volumes of 1-bromo-3-chloropropane (BCP). The prep is then centrifuged, which results in phase separation with the RNA contained in the upper aqueous phase. The aqueous phase is recovered and mixed with ethanol and magnetic beads, and incubated with shaking at room temp or at 55°C (without agitation) to bind the RNA to the beads. The beads are then magnetically attracted, supernatant is removed, and beads are washed with 80% ethanol. The RNA is then eluted from the beads in the included Elution Solution or in RNase-free water or other solvent of choice. The extraction procedure can be completed in less than one hour.

Contents, Storage and Shelf Life

The NextPrep™ Magnazol™ cfRNA Isolation Kit contains reagents for processing up to 25 samples of 0.6 mL of plasma or serum. The Kit is stable for at least one year when properly stored.

Kit Contents	Amount	Storage
Magnazol™ Extraction Reagent	30 mL	4°C
Magnazol™ Magnetic Beads	1.3 mL	4°C
BCP (1-bromo-3-chloropropane)	3 mL	Room Temp.
RNA Elution Solution (0.1 mM EDTA)	1 mL	Room Temp.

Revision History

Version	Date	Description
V18.05	May 2018	Initial Product Launch.
V19.03	March 2019	Updated language of protocol for additional clarity.

Required Materials Not Provided

- Ethanol, 200 proof, without added ketones, other alcohols, or other chemical denaturants
- 80% ethanol made in nuclease-free water
- 2 mL microcentrifuge tubes; 1.7 mL microcentrifuge tubes are NOT recommended for use with a standard 0.6 mL prep
- Pipettors and tips
- Microcentrifuge capable of reaching 16,000g
- Platform shaker, tube rotator, or heat block
- Magnetic stand for 2 mL microcentrifuge tubes, for example cat. # 12321D from Thermo Fisher Scientific®

Warnings and Precautions

The Magnazol™ Extraction Reagent contains phenol and guanidinium salts and can cause severe injury if it comes into contact with eyes or skin. Wear safety goggles and use personal protective equipment when using this product. If the Magnazol™ Extraction Reagent comes into contact with skin or eyes, wash with large amounts of water and seek medical advice. Do not mix solutions containing Magnazol™ Extraction Reagent with bleach. Refer to the MSDS for more information on hazards associated with this product.

1. Allow the Magnazol™ Extraction Reagent and magnetic beads to warm to room temp before use. For standard prep: In a 2 mL microcentrifuge tube, mix 0.6 mL plasma or serum with 1.2 mL Magnazol™ Extraction Reagent. Shake and/or vortex vigorously for ~ 5 seconds. See Table 1 (Appendix B) for volumes of Magnazol™ Extraction Reagent and BCP to use for sample volumes that differ from the standard 0.6 mL prep.
2. Add 120 µL of BCP (1-bromo-3-chloropropane) and mix by vigorous vortexing for ~10 seconds to create an emulsion. It is important to thoroughly mix the BCP with the sample.
3. Spin the prep for 5 minutes at 16,000g at room temp or 4°C.
4. Remove the aqueous phase (top phase, which should be colorless) to a new 2 mL tube. The volume of aqueous phase is typically about 400 µL, or about two-thirds the volume of plasma or serum used. **Use care to ensure that the aqueous phase is not contaminated by any material from the organic phase. Leave a small amount of aqueous phase behind as necessary to avoid contamination with organic phase.**
5. Thoroughly mix the aqueous phase with 2.5 volumes of ethanol. Typically, this is 1 mL of ethanol.
6. Vortex the magnetic beads and examine to verify that bead mixture is homogeneous before use. Add 50 µL of well-mixed magnetic beads to the prep and mix thoroughly.
7. Incubate the prep for 10 minutes at room temperature with agitation on a platform shaker. Typical shaking speed is 1,500 rpm. Alternatively, the prep can be mixed using a tube rotator, or incubated without agitation in a heat block at 55°C.
8. Briefly spin the tube to collect all liquid (including liquid from the inside of the lid), then place the tube on a magnetic stand for 1 minute or as needed to thoroughly attract the beads.
9. Remove and discard the supernatant fluid. Leave the tube on the magnet for subsequent washing steps.
10. Wash beads with 1.5 mL 80% ethanol, flowing it down the side of the tube but avoiding flowing it over the attracted beads. Wait ~20 seconds and remove wash solution.
11. Repeat previous step, for a total of two 80% ethanol wash steps.
12. Remove residual wash solution by briefly spinning the tube to collect the liquid, returning the tube to the magnet, and removing the collected liquid. Alternatively, residual wash solution may be removed by drying the open tube for ~ 3 minutes, tapping the magnetic stand against a hard surface several times to collect the residual fluid at the bottom of the tube and removing it.
13. Resuspend the beads in RNA Elution Solution (or other solvent of choice). Recommended minimum elution volume is 16 - 18 µL for preps using the standard 0.6 mL of sample. Inspect side of tube to ensure bead pellet is completely resuspended.
14. Store prep for 3 minutes at room temperature with shaking or in a heat block at 55°C.
15. Briefly spin the tube to collect bead slurry then place tube on magnetic stand for ~ 1 minute to attract beads, then transfer eluate containing the recovered RNA to a new tube.

Troubleshooting

Recovery of RNA may be compromised by procedural details such as:

- Failure to thoroughly mix the sample with the Magnazol™ Extraction Reagent
- Failure to thoroughly mix the prep with the BCP
- Inadequate mixing of the aqueous phase with ethanol or failure to use 2.5 volumes of 100% ethanol per volume of aqueous phase
- Incomplete attraction of magnetic beads, especially during the initial attraction step
- Incomplete resuspension of magnetic beads in the elution solution

Alternative Starting Volumes

NextPrep™ Magnazol™ cfRNA Isolation Kit was tested and optimized with a starting volume of 0.6 mL serum or plasma, the highest volume that can be processed in a 2 mL tube. Extraction from other volumes may require some optimization. Bead volumes in particular may not scale proportionally, with a greater proportion of beads needed for higher volume preparations. Refer to Table 1 for volumes of Magnazol™ Extraction Reagent and BCP to use with different serum/plasma input volumes.

Component	Volume to add relative to plasma or serum volume	Example for 0.2 mL plasma or serum	Example for 0.5 mL plasma or serum	Example for 0.6 mL plasma or serum	Example for 1 mL plasma or serum*	Example for 1.2 mL plasma or serum*
Magnazol™ Extraction Reagent	2X	0.4 mL	1 mL	1.2 mL	2 mL	2.4 mL
BCP	0.2X	40 µL	100 µL	120 µL	200 µL	240 µL

Table 1. Volumes of Magnazol™ Extraction Reagent and BCP to use for processing various volumes of plasma or serum, up to 1.2 mL

*** Note, sample volumes greater than 0.6 mL will need to be split into multiple microcentrifuge tubes.**

Examples of RNA Recovery

Shown below are representative Agilent® Bioanalyzer® Small RNA Assay traces of RNA recovered from 0.6 mL of platelet-rich plasma, platelet-poor plasma, and serum. Serum and platelet-poor plasma often have RNA content too low to be detected by this assay; however, **Small RNA-Seq libraries can usually be made from these samples using the NEXTFLEX® Small RNA Sequencing Kit v3** with the low-input protocol.

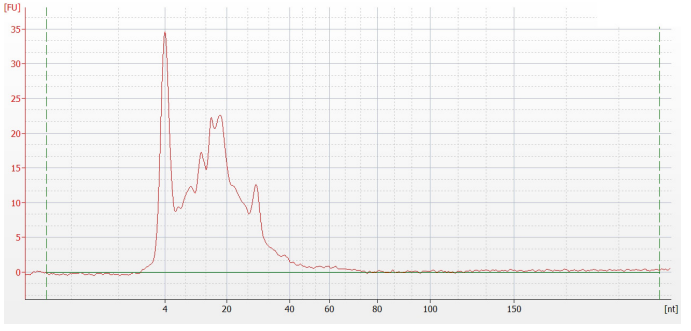

Figure 1. Representative example of RNA recovery from 0.6 mL of platelet-rich plasma, eluted in 16 µL RNA Elution Solution.

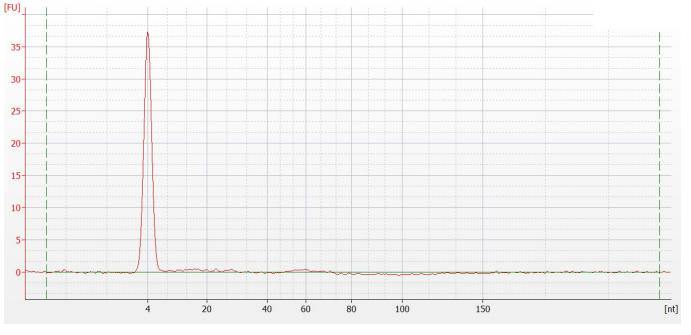

Figure 2. Representative example of RNA recovery from 0.6 mL of platelet-poor plasma, eluted in 16 µL RNA Elution Solution.

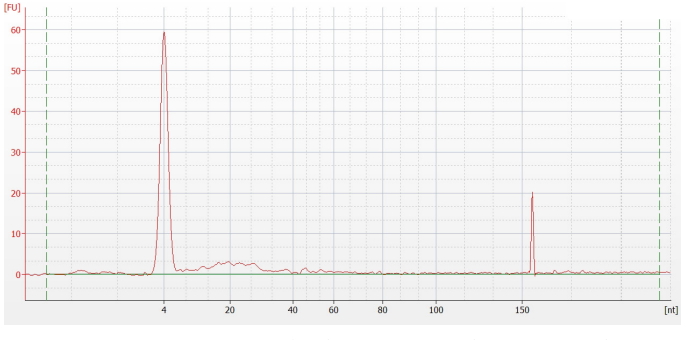

Figure 3. Representative example of RNA recovery from 0.6 mL of serum, eluted in 16 µL RNA Elution Solution.

NOTES

WE WANT TO HEAR FROM YOU!

Your feedback is important to us. Tell us what you think of our kits by scanning the QR code or visiting our website at www.biooscientific.com/NGSfeedback. We can't wait to hear from you!

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