NZY Stool RNA Isolation Kit

Catalogue number: MB45701, 50 columns

Description

The NZY Stool RNA Isolation kit is engineered to ensure efficient extraction of RNA, including smaller RNA species, from both fresh and frozen stool samples. Utilizing the combined action of Buffer NST NZYol and a specialized Lysis Buffer NSTL, the kit offers an effective chemical disruption method for the stool sample and the microbes contained therein. This chemical disruption is supplemented by mechanical lysis using NZYSpin Stool Bead Tubes (equipped with ceramic beads) and a 10-minute shaking on a Vortex. Importantly, the homogenization of the sample material does not necessitate any enzymatic reactions such as protease digestion. The kit's procedure involves separating the undissolved sample material and ceramic beads through brief centrifugation. This is followed by adding Buffer NSTB (binding buffer) to further clear the lysate. The clarified supernatant is subsequently passed through the NZYSpin RNA Stool Filter Columns (red rings), effectively eliminating elements from stool samples that could hinder RT-PCR processes. After adjusting the binding conditions by introducing additional Buffer NSTB to the NZYSpin RNA Stool Filter Columns flow-through, the sample is loaded onto NZYSpin RNA Stool Columns (light blue rings). Any lingering contaminants, including complex polysaccharides, bile salts, and other PCR inhibitors, are then effectively removed through a thorough washing procedure utilizing Buffer NSTB along with Wash Buffers NSTW1, NSTW2, and NSTW3. Residual DNA is eliminated during the washing stages via an on-column DNA digestion process involving rDNase. Following a drying phase, the RNA, now ready for use, can be eluted using RNase-free water. Furthermore, the NZY Stool RNA Isolation Kit allows isolating various nucleic acid compositions from stool samples. The standard protocol is designed to isolate total RNA, inclusive of smaller RNA species. If DNA extraction from the stool sample is required, the on-column DNA digestion step can be omitted. In situations where small RNA

species are desired, the total quantity of Buffer NSTB for the binding step can be reduced. The procedure involving the NZY Stool RNA Isolation Kit can be conducted at room temperature. However, it is essential to handle the resulting eluate with caution due to RNA's high sensitivity to trace RNase contaminations, commonly found on laboratory equipment, fingerprints, and dust. To maintain RNA stability, it is recommended to store the RNA at -20 °C for short-term preservation or -70 °C for long-term storage.

Starting Stool sample

The NZY Stool RNA Isolation Kit is designed for optimal performance with 180–220 mg (approximately 200 mg) of human stool. However, when processing animal stool samples, reducing the sample quantity might yield superior outcomes. Dry stool samples, typical of rabbits or mice, may absorb the lysis buffer, causing an inadequate sample volume following the first centrifugation step. In these situations, it is advisable to decrease the stool material to around 30–80 mg and to increase the volume of the lysis reagents (see the section below for further details regarding input material). For more challenging stool samples, which may be rich in lipids, polysaccharides, or proteins, minimizing the starting material can potentially enhance lysis efficiency and improve RNA purity. For such samples, it is recommended to initiate the extraction with 50–100 mg of sample material. Human stool samples can sometimes contain undigested food particles (like crop or fruit husks, and undigested seeds). These particles should be avoided during transfer to the NZYSpin RNA Stool Columns. The best results are typically achieved with fresh sample material, ideally stored at 2–8 °C after collection and used within 24 hours for RNA extraction. If immediate extraction isn't possible, freeze the stool sample as soon as possible after collection at -20 °C. The thawing process should be performed at room temperature just prior to extraction, or overnight on ice. Avoid multiple freeze-thaw cycles, as these can lead to RNA degradation.

Handling and general recommendations

Effective sample lysis is crucial for obtaining high RNA yield and eliminating contaminants during the silica column purification process. Given the complex composition of stool samples, which include food residues, lipids, proteins, bile salts, and polysaccharides, chemical lysis facilitated by Buffer NST NZYol and Lysis Buffer NSTL is complemented by mechanical disruption in the NZYSpin Stool Bead Tubes. This dual approach ensures comprehensive solubilization of the sample material. The ten-minute shaking process on a Vortex effectively suspends even solid stool samples like mouse droppings, causing both host and microbial cells to rupture. For animal stool samples, the standard protocol may not yield optimal results. A general suggestion for all animal stool samples is to use 150 µL of Buffer NST NZYol and, depending on the stool sample's water content, 700 to 800 µL of Lysis Buffer NSTL for the sample lysis step. This ensures a minimum of 500 µL of lysate can be transferred following the initial centrifugation step. Furthermore, the starting material amount should be lower compared to human stool samples. For carnivores (such as cats) and birds (such as chickens), it's recommended to use a maximum of 100 mg of stool sample. For fiber-rich, dry stool samples from herbivores (like rabbits or sheep), use between 50–80 mg. For extremely hard and dry samples, such as dried mouse droppings, only 30–40 mg of stool should be used as the starting material. Optimal RNA yield and purity can be achieved from animal stool samples by adjusting the conditions for lysate clearing and RNA binding. Refer to **Table 1** for recommendations specific to select species.

Table 1. Suggested NSTB Volumes for Lysate Clearing and RNA Binding Steps.

Stool sample source	Recommended NSTB Volume for Lysate Clearing	Recommended NSTB Volume for RNA Binding Step
Feline, Sheep, Rabbit	200 µL	170 µL
Mouse, Chicken	140 µL	240 µL

Please note that these are general guidelines and may need to be modified based on the specific condition and quality of your samples.

The washing procedure has been meticulously calibrated to efficiently eliminate any residual contaminants bound to the silica membrane. This comprehensive process begins with a wash using Wash Buffer NSTW1, succeeded by an on-column DNase digestion. If you aim to isolate DNA as well, skip the DNase digestion step. The second wash utilizes Wash Buffer NSTW2, which is followed by two additional washing steps employing Buffer NSTB and the final Wash Buffer NSTW3. After the final centrifugation to thoroughly dry the silica membrane RNA can be eluted using RNase-free water.

We recommend using 100 µL of RNase-free water for the elution process (**Table 2**). If you choose to use a volume less than 100 µL for elution, ensure to pipette the RNase-free water directly onto the center of the column to fully moisten the silica membrane.

Please note that using a volume less than 100 µL for the elution step might lead to a decrease in the overall yield. Using less than 30 µL of RNase-free water for elution is strongly discouraged. If you opt for less than 60 µL for the elution, the yield could be improved by applying the elution buffer twice onto the spin column. After the initial elution step, re-pipette the eluate from the elution tube back onto the membrane of the NZYSpin RNA Stool Column (light blue rings) and repeat the centrifugation for 1 minute at 13,000 x g.

Table 2. Elution volume recommendations for RNA Isolation.

Elution Volume	Recommendations
100 µL	Optimal volume.
<100 µL	May reduce yield; Ensure to moisten the silica membrane completely.
<60 µL	Apply the elution buffer twice onto the spin column to improve yield.
<30 µL	Strongly discouraged due to significant potential reduction in yield.

Storage conditions and reagents preparation

Upon arrival, store the lyophilized rDNase (RNase-free) at 4 °C (stable for up to 1 year). All other kit components should be stored at 15–25 °C and are stable for at least one year. Storage at lower temperatures may lead to salt precipitation. If this occurs, warm the bottle for a few minutes at approximately 30–40 °C and stir thoroughly until the precipitate dissolves.

Before starting the first NZY Stool RNA Isolation procedure, prepare the Wash Buffer NSTW3 as follows: Add 48 mL of ethanol (96–100%) to 12 mL of concentrate Buffer NSTW3. Indicate on the bottle label that ethanol has been added. Buffer NSTW3 is stable at room temperature (15–25 °C) for at least one year.

To prepare rDNase (RNase-free), add 1.4 mL Digestion Buffer to each rDNase vial and allow to sit for 1 min at room temperature. Gently swirl the vials to fully dissolve the rDNase but avoid vigorous shaking as rDNase is sensitive to mechanical agitation. Divide the rDNase solution into aliquots in nuclease-free 1.5 mL microcentrifuge tubes (not provided) and store at -20 °C. The frozen working solution will be stable for at least 6 months. Avoid more than three freeze-thaw cycles for the aliquots.

Note: Be cautious when opening the rDNase vial as some particles of the lyophilizate may be stuck to the lid. In some instances, the rDNase vial might seem empty due to the lyophilized enzyme sticking to the septum. To prevent loss of rDNase, ensure to collect it at the bottom of the vial before removing the plug. Alternatively, inject RNase-free water into the vial using a needle and syringe, invert the vial to dissolve the rDNase, and remove the dissolved rDNase using the same syringe and needle.

Safety Alert: Buffer NST NZYol includes phenol (a corrosive and toxic liquid) and guanidium thiocyanate (an irritant). It is essential to use personal protective equipment, including gloves, eye protection, and safety goggles, while handling these substances.

Caution: Please refer to the warning label on the container and the Material Safety Data Sheet (MSDS). Buffer NST NZYol, which contains phenol and guanidinium thiocyanate, can cause burns and even be fatal. While handling Buffer NST NZYol, ensure to wear gloves and eye protection (a face shield or safety goggles). Avoid contact with skin or clothing and refrain from inhaling fumes. In case of accidental contact, immediately rinse the affected area with plenty of water for at least 15 minutes and seek medical attention if necessary.

Components

Component	Volume (50 preps)
Buffer NST NZYol	12 mL
Buffer NSTL	40 mL
Buffer NSTB	50 mL
Buffer NSTW1	36 mL
Buffer NSTW2	35 mL
Buffer NSTW3 (concentrate)	12 mL
RNase-free Water	13 mL
Digestion buffer	7 mL
rDNAse (lyophilized)	3 vials
NZYSpin Stool Bead Tubes	50
NZYSpin RNA Stool Filter Columns (red rings)	50
NZYSpin RNA Stool Columns (light blue rings)	50
Collection tubes (2 mL)	50
Collection tubes (2 mL, lid)	50

Guidelines for using NZY Stool RNA Isolation Kit

Before beginning the preparation, ensure the following steps are taken:

• Inspect Lysis Buffer NSTL for any precipitates. If any are present, dissolve them by warming the buffer at a temperature between 40–50 °C.

• Prepare the rDNase solution with rDNase that has been reconstituted in the Digestion buffer. Alternatively, thaw the necessary aliquots from the -20 °C freezer and maintain the rDNase on ice until it's ready for use.

• For each RNA extraction, 80 µL of the reconstituted rDNase – rDNAse solution will be utilized.

General Protocol

1. **Sample Preparation:** Refer to the sections above for guidance on starting material volume and lysis procedures for different species' stool samples.

   Transfer 180–220 mg of human stool material to a NZYSpin Stool Bead Tube, ensuring not to overload the tube. Combine 200 µL Buffer NST NZYol and 660 µL Buffer NSTL. For animal stool samples, adjust the volume accordingly (refer to the sections above for details).

2. **Sample Lysis:** Agitate the NZYSpin Stool Bead Tube using a suitable disruption device. Centrifuge the tube for 5 min at 13,000 *x g* and transfer 510 µL of the supernatant to a fresh 2 mL microcentrifuge tube.

3. **Contaminant Precipitation:** Add 140 µL Buffer NSTB, vortex briefly, and centrifuge for 3 min at 13,000 *x g*. Note that the volume of NSTB may need adjustment for animal stool samples (see section above, **Table 1**).

4. **Lysate Filtration:** Place a NZYSpin RNA Stool Filter Column (red ring) in a Collection Tube (2 mL, lid) and transfer 600 µL of the cleared lysate onto the column. Centrifuge for 1 min at 13,000 *x g* and discard the column.

5. **Binding Condition Adjustment:** Add 180 µL Buffer NSTB (for total RNA) or 120 µL Buffer NSTB (for large RNA only) and vortex briefly.

6. **RNA Binding:** Place a NZYSpin RNA Stool Column (light blue ring) in a Collection Tube (2 mL) and load 600 µL of the sample onto the column. Centrifuge for 1 min at 13,000 *x g*, discard the flowthrough, reload the residual sample, and repeat the centrifugation.

7. **Silica Membrane Washing and DNA Digestion:** Perform four wash steps using 600 µL of Buffers NSTW1, NSTW2, NSTB, and NSTW3 respectively, centrifuging for 1 min at 13,000 *x g* between each wash. In the second wash step, apply 80 µL rDNase solution directly onto the column's silica membrane and incubate at room temperature for 15 min. Skip this step if co-purifying DNA with RNA.

8. **Silica Membrane Drying:** Following the washes, centrifuge the column for 2 min at 13,000 *x g* to dry the silica membrane. Repeat this step if the liquid in the collection tube contacts the column.

9. **RNA Elution:** Transfer the NZYSpin RNA Stool Column (light blue ring) to a new nuclease-free 1.5 mL microcentrifuge tube, add 100 µL RNase-free water, and centrifuge for 1 min at 13,000 *x g* to elute the RNA. If using a lower elution volume, refer to the section above (**Table 2**) for recommendations. Discard the column and briefly vortex the microcentrifuge tube.

Remember to wear appropriate personal protective equipment throughout the procedure. It's recommended to use a balance to weigh sample material, aspirate the supernatant carefully, and avoid adding bleach or acidic.

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