MD0800_IFU_EN_V2501

# NZY Mag NGS Clean-up & Size Selection

| Catalogue number | Presentation |
|---|---|
| MD08001 | 5 mL |

## Description

The NZY Mag NGS Clean-up & Size Selection is a cutting-edge paramagnetic bead-based system optimized for rapid and efficient purification of DNA and RNA fragments from enzymatic reactions, as well as precise size selection of DNA during the library construction process for next-generation sequencing (NGS) workflows. This solution is formulated to provide scientists with high recovery yields and exceptional flexibility, addressing common challenges in clean-up and size selection steps. Utilizing NZYtech's proprietary paramagnetic bead suspension in a specialized binding buffer, nucleic acids are selectively bound based on the ratio of beads to sample. The workflow includes:

- Binding of DNA or RNA to the beads.
- Magnetic separation and removal of contaminants (nucleotides, primers, adapters, dimers, enzymes, salts, and buffer additives).
- Ethanol washes to ensure thorough purification.
- A short drying step to eliminate residual ethanol.
- Elution of highly pure nucleic acids, RNA or DNA, in a low-salt elution buffer or water for immediate downstream applications.

The purified DNA and RNA are suitable for a wide range of molecular biology and sequencing applications, including PCR amplification, cDNA synthesis, RNA-Seq, whole-genome sequencing, and targeted sequencing workflows. The NZY Mag NGS Clean-up & Size Selection was designed for manual or automated purification of DNA and RNA fragments from various enzymatic reaction mixtures, such as:

- Fragmentation reactions
- End-repair reactions
- A-tailing reactions
- Adapter ligation mixtures
- PCR amplification products
- cDNA synthesis reactions
- *In vitro* transcription products
- RNA amplification workflows

The NZY Mag NGS Clean-up & Size Selection delivers exceptional recovery and precision, achieving typical yields of > 80% for DNA and > 70% for RNA, while enabling tunable size selection of double-stranded DNA fragments between 150 bp and 800 bp with input amounts ranging from 5 ng to 1 µg. Designed for optimized reproducibility, the system accommodates sample volumes of ≥ 50 µL and operates seamlessly at room temperature, eliminating the need for refrigeration. Its versatility extends to manual workflows and automated liquid-handling systems, offering scalable solutions for high-throughput applications. The resulting highly purified nucleic acids are of unparalleled quality, supporting critical downstream processes such as PCR, qPCR, NGS library preparation, sequencing on all major platforms, *in vitro* transcription, and other molecular analyses. By combining precision, adaptability, and automation compatibility, the NZY Mag NGS Clean-up & Size Selection ensures superior performance for the demanding clean-up and size selection requirements of modern NGS workflows.

## Shipping and Storage conditions

The NZY Mag NGS Clean-up & Size Selection is shipped at room temperature. Upon arrival, the bead suspension must be stored at 2–8 °C, ensuring stability until the expiry date indicated on the package label. The NZY Mag NGS Bead Clean-up & Size Selection is supplied ready to use, requiring no additional preparation before use, providing a seamless and efficient workflow for clean-up and size selection processes.

## Components

| COMPONENT | MD08001 |
|---|---|
| NZY Mag NGS Bead Solution | 5 mL |

## Reagents, Materials and Equipment Required but Not Provided

- 80% ethanol
- Elution buffer (10 mM Tris-HCl, pH 8.0, RNase-free) or RNase-free water
- Disposable pipette tips (Sterile, RNase-free, and filtered tips recommended)
- Well-calibrated pipettors for accurate liquid handling
- Vortex mixer for bead resuspension
- Magnetic separation system, such as a 96-well magnetic stand compatible with paramagnetic bead workflows
- Separation plate for magnetic bead-based separations, e.g., 96-well 0.3 mL U-bottom microtiter plates
- Plate seals to prevent evaporation, e.g., self-adhering polyethylene (PE) foil.

## Handling of beads and magnetic separation systems

The NZY Mag NGS Clean-up & Size Selection is designed for optimal performance when combined with appropriate magnetic separation systems. For manual workflows, NZYtech recommends using a 96-well magnetic stand or other commonly available systems, such as ring static magnetic stands, compatible with microtiter plates or tubes. Separation is performed in standard U-bottom 96-well plates (300 µL wells) or alternative formats suitable for the workflow.

Precise handling of the NZY Mag NGS Bead Solution is critical to ensuring reliable results, particularly for size selection applications where accuracy directly influences fragment distribution. To achieve this:

- Use calibrated pipettes and change tips between samples (single-channel) or columns (multi-channel pipettes).
- Handle the slightly viscous bead suspension with care: aspirate and dispense slowly to avoid introducing air bubbles and ensure complete transfer. Avoid droplets on the exterior of the pipette tip.
- Prior to use, the bead suspension must be homogenized for consistent results across wells. Briefly vortex the storage bottle or shake thoroughly to resuspend the beads evenly.

The efficiency of magnetic separation depends on several factors: the magnetic strength, the distance between the separation plate and the magnetic field, and the volume of the sample being processed. Optimization of separation time for specific systems is recommended to ensure complete bead attraction. Use plates or tubes as specified by the magnetic stand manufacturer for best performance. The NZY Mag NGS Bead Solution allows tunable size selection of DNA fragments based on the bead-to-sample volume ratio, enabling the recovery of specific size ranges tailored to sequencing requirements. By adjusting the volume ratio:

- Increasing the bead volume relative to the sample promotes the binding of shorter fragments.
- The solution supports the recovery of double-sided or single-sided size selection workflows, achieving libraries with fragment sizes between 150 bp and 800 bp, ideal for NGS applications such as Illumina sequencing.

The system is fully compatible with standard NGS library preparation protocols, allowing users to employ bead-to-sample ratios identical to those recommended by their library preparation kits. By combining precise handling, customizable size selection, and streamlined magnetic separation, the NZY Mag NGS Clean-up & Size Selection ensures exceptional accuracy, consistency, and flexibility for high-quality library construction workflows.

## Standard Protocol

**Recommendations before starting**

- Remove the NZY Mag NGS Bead Suspension from 2–8 °C storage and allow it to equilibrate to room temperature (~30 minutes). Vortex the bead suspension thoroughly to ensure a homogeneous solution.

**Procedures before starting**

The NZY Mag NGS Bead Suspension has been rigorously tested to ensure they are RNase-free. However, maintaining an RNase-free work environment is essential for the successful clean-up and handling of RNA. To prevent RNase contamination, the following precautions are strongly recommended:

- Work in a dedicated RNA handling area with separate pipettors and materials specifically reserved for RNA workflows.
- Wear gloves at all times to avoid skin contact, a common source of RNases, and change gloves frequently during the process.
- Use only sterile, RNase-free plastic consumables to prevent contamination.
- Ensure elution is performed with RNase-free water.
- Keep all kit components sealed when not in use and tightly close all tubes whenever possible to minimize exposure.

By adhering to these recommendations, the integrity of RNA samples can be preserved, ensuring consistent and reliable results in clean-up workflows.

## Protocol for DNA clean-up and single-size selection

**1. Bind Target DNA to Beads**

i. Vortex the NZY Mag NGS Bead Solution thoroughly.
ii. Add 100 µL of NZY Mag NGS Bead Solution to a well or tube.
iii. Add 100 µL of DNA sample (1:1 ratio of bead suspension to sample).
iv. Mix well by pipetting up and down 10 times using a calibrated pipette set to 200 µL.
v. Incubate at room temperature for 5 minutes to allow DNA binding.
vi. Place the plate/tube on the magnetic stand. Wait 5 minutes or until the liquid is clear.
vii. Carefully remove and discard the supernatant without disturbing the magnetic bead pellet.

**Note:** *The supernatant contains smaller DNA fragments and contaminants. Aspirate from the side opposite the beads.*

**2. Wash Beads (1st Ethanol Wash)**

i. With the plate/tube on the magnetic stand, add 200 µL of 80% ethanol (not provided) without disturbing the beads.
ii. Incubate for 30 seconds at room temperature.
iii. Carefully remove and discard the ethanol.

**3. Wash Beads (2nd Ethanol Wash)**

i. Repeat the ethanol wash step: Add 200 µL of 80% ethanol (not provided), incubate for 30 seconds, and remove the ethanol completely.
ii. Ensure all ethanol, including residual droplets, is removed.

**4. Dry the Beads**

i. Leave the plate/tube on the magnetic stand.
ii. Allow the beads to air-dry for 5–15 minutes at room temperature until residual ethanol evaporates.

**Note:** *Avoid overdrying the beads, as it may reduce DNA yield. The pellet should appear dry with no visible liquid droplets.*

**5. Elute DNA**

i. Remove the plate/tube from the magnetic stand.
ii. Add 10–50 µL of elution buffer (e.g., 10 mM Tris-HCl, pH 8, or RNase-free water).
iii. Resuspend the beads by:
    - Pipetting up and down (10 times), or
    - Shaking at 1100 rpm (e.g., Eppendorf Thermomixer®).
iv. Incubate at room temperature for 2–5 minutes to elute DNA.
v. Return the plate/tube to the magnetic stand. Wait 5 minutes or until the liquid is clear.
vi. Carefully transfer the supernatant containing the purified DNA to a new plate or tube.

**Next Steps:** *Proceed to the next step of your NGS library preparation workflow using the purified DNA fragment library*.

This protocol ensures high recovery, precision, and reproducibility for clean-up and size selection, meeting the stringent requirements of modern NGS workflows.

## Protocol for removing adapter dimers

This protocol outlines the removal of **adapter dimers** following an adapter ligation reaction using two successive purification steps. The first step exchanges the ligation buffer to eliminate interfering agents, while the second step selectively removes adapter dimers.

**1. Exchange Ligation Reaction Buffer**

i. Perform the purification procedure as described in the standard clean-up protocol (Section Protocol for DNA clean-up and single-size selection) using a bead-to-sample ratio of **1.0**.
    - Example: For 50 µL of ligation reaction, add 50 µL of NZY Mag NGS Bead Solution.
ii. Elute the purified DNA in 50 µL of elution buffer (e.g., 10 mM Tris-HCl, pH 8, or RNase-free water).

**2. Remove Adapter Dimers**

i. Using the 50 µL eluate from Step 1, perform the purification procedure again with a bead-to-sample ratio of **0.8**.
    - Example: Add 40 µL of NZY Mag NGS Bead Solution to the 50 µL eluate.
ii. Elute the DNA in 30 µL of elution buffer.

**Next Steps:** *Proceed to the next step of your library construction workflow using the adapter-dimer-free DNA fragments.*

This two-step process effectively removes unwanted adapter dimers and ensures clean, high-quality DNA for downstream applications, supporting robust and reliable NGS library preparation.

## Protocol for DNA double-size selection

This protocol enables the generation of DNA fragment libraries within a defined size range by removing both smaller and larger fragments in two successive steps. For example, the following procedure demonstrates the selection of DNA fragments in the **400–500 bp** range. Adjusting the bead-to-sample volume ratios allows for other size ranges as needed.

**1. Remove Unwanted Larger DNA Fragments (Right-Side Selection)**

i. Vortex the NZY Mag NGS Bead Solution thoroughly.
ii. Add 40 µL of NZY Mag NGS Bead Solution to 100 µL of DNA sample (**ratio = 0.4**).
iii. Mix by pipetting up and down (10 times, set pipette to 140 µL).
iv. Incubate at room temperature for 5 minutes to allow binding of larger fragments.
v. Place the plate/tube on the magnetic stand. Wait 5 minutes or until the liquid is clear.
vi. Carefully transfer the supernatant (containing smaller fragments) to a new plate or tube.
vii. Discard the beads, which contain unwanted larger DNA fragments.

**2. Remove Unwanted Smaller DNA Fragments (Left-Side Selection)**

i. Vortex the NZY Mag NGS Bead Solution again until homogeneous.
ii. Add 20 µL of NZY Mag NGS Bead Solution to the supernatant from Step 1 (total bead-to-sample ratio = **0.6**).
iii. Mix by pipetting up and down (10 times, set pipette to 160 µL).
iv. Incubate at room temperature for 5 minutes to bind fragments above the lower size limit.
v. Place the plate/tube on the magnetic stand. Wait 5 minutes or until the liquid is clear.
vi. Carefully remove and discard the supernatant without disturbing the magnetic beads.

**3. Wash Beads (1st Ethanol Wash)**

i. With the plate/tube on the magnetic stand, add 200 µL of 80% ethanol (not provided).
ii. Incubate at room temperature for 30 seconds.
iii. Carefully remove and discard the ethanol without disturbing the beads.

**4. Wash Beads (2nd Ethanol Wash)**

i. Repeat the ethanol wash: Add 200 µL of 80% ethanol (not provided).
ii. Incubate at room temperature for 30 seconds.
iii. Completely remove and discard the ethanol, ensuring no residual droplets remain.

**5. Dry the Beads**

i. Leave the plate/tube on the magnetic stand and allow the beads to air-dry for 5–15 minutes at room temperature.
ii. Ensure the pellet is dry, with no visible liquid, but avoid over-drying, as this may reduce DNA recovery.

**6. Elute DNA Fragment Library**

i. Remove the plate/tube from the magnetic stand.
ii. Add 10–50 µL of elution buffer (e.g., 10 mM Tris-HCl, pH 8.0, or RNase-free water).
iii. Resuspend the beads by:
    a. Pipetting up and down (10 times), or
    b. Shaking at 1100 rpm (e.g., Thermomixer®).
iv. Incubate at room temperature for 2–5 minutes.
v. Return the plate/tube to the magnetic stand. Wait 5 minutes or until the liquid is clear.
vi. Carefully transfer the supernatant (containing the purified DNA library) to a new plate or tube.

**Next Steps:** Proceed with the next step of your NGS library preparation workflow.

This double-size selection method effectively removes unwanted fragments, delivering DNA libraries with a narrow and defined size range essential for high-quality sequencing applications.

### Protocol for PCR, DNA or RNA clean-up

This protocol efficiently removes contaminants such as nucleotides, primers, adapters, enzymes, salts, and buffer additives from enzymatic reaction mixtures. A **1.8:1 bead-to-sample volume ratio** ensures optimal binding of nucleic acids. The protocol is compatible with standard 96-well PCR plates and appropriate magnetic stands (e.g., ring-magnet systems).

**1. Bind Target DNA or RNA to Beads**

i. Vortex the NZY Mag NGS Bead Suspension thoroughly until it appears homogeneous.
ii. Add 1.8 volumes of bead suspension to each well containing the sample (refer to the table below for suggested volumes):

| SAMPLE REACTION VOLUME | NZY MAG NGS BEAD SOLUTION VOLUME |
|---|---|
| 10 µL | 18 µL |
| 20 µL | 36 µL |
| 50 µL | 90 µL |
| 100 µL | 180 µL |

**Note**: *For volumes larger than 200 µL, use a larger container, such as a deep-well plate.*

iii. Mix thoroughly by pipetting up and down (10 times) or by shaking until the mixture appears homogenous.
iv. Incubate at room temperature for 5 minutes to allow nucleic acid binding.
v. Place the plate on a magnetic stand and wait for 2 minutes until the beads are completely attracted to the magnet and the liquid appears clear.
vi. Carefully remove and discard the supernatant without disturbing the bead pellet.

**2. Wash Beads (1st Ethanol Wash)**

i. Leave the plate on the magnetic stand.
ii. Add 200 µL of 80% ethanol (not provided) to each well without disturbing the bead pellet.
iii. Incubate at room temperature for 30 seconds.
iv. Carefully remove and discard the ethanol.

**3. Wash Beads (2nd Ethanol Wash)**

i. Repeat the ethanol wash: Add 200 µL of 80% ethanol (not provided) to each well.
ii. Incubate at room temperature for 30 seconds.
iii. Carefully remove and discard the ethanol, ensuring all residual droplets are removed.

**4. Dry the Beads**

i. Leave the plate on the magnetic stand and allow the beads to air-dry for 5–15 minutes at room temperature.
ii. Ensure the pellet is dry with no visible liquid but avoid overdrying as this may reduce recovery of longer nucleic acid fragments.

**5. Elute DNA or RNA**

i. Remove the plate from the magnetic stand.
ii. Add 10–50 µL of elution buffer (e.g., 10 mM Tris-HCl, pH 8.0, or RNase-free water).

**Note:** *For higher expected yields, larger elution volumes (e.g., 100 µL) can be used.*

iii. Resuspend the bead pellet by pipetting up and down (10 times).
iv. Incubate at room temperature for 2–5 minutes to facilitate elution.
v. Return the plate to the magnetic stand and wait for 2 minutes or until the liquid is clear.
vi. Carefully transfer the supernatant containing the purified DNA or RNA to a new plate or tube.

**Next Steps:** Proceed with the downstream application of your purified nucleic acid.

This protocol ensures reliable removal of contaminants and high recovery of nucleic acids, making it ideal for clean-up workflows in NGS library preparation, PCR, cDNA synthesis, and other enzymatic processes.

## Quality control assay

NZY Mag NGS Clean-up & Size Selection is tested following standard protocols.

## Troubleshooting

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| **POOR DNA/RNA YIELD** |
| • **Insufficient bead-to-sample ratio** |
| Use the recommended volume ratios outlined in this manual (e.g., 1.0). |
| • **Ethanol concentration too low** |
| Use freshly prepared 80% ethanol to prevent DNA loss caused by ethanol evaporation or dilution. |
| • **Insufficient elution buffer volume** |
| Ensure the bead pellet is completely covered with elution buffer. |
| • **Short elution incubation** |
| Incubate beads in elution buffer for at least 5 minutes to optimize recovery. |
| **LOW RNA RECOVERY / DEGRADED RNA** |
| • **RNase contamination** |
| Maintain an RNase-free work environment:<br>- Wear gloves during all steps and change them frequently.<br>- Use RNase-free consumables and keep tubes/plates closed when possible.<br>- Oven-bake glassware for 2 hours at 250 °C or treat with RNase decontamination solutions. |
| **UNEXPECTED RATIO $A_{260}/A_{280}$** |
| • **RNase contamination** |
| RNA base composition affects absorbance ratios. For example, Poly-A+ RNA typically shows:<br>- $A_{260}/A_{280}$: 3.3–3.7<br>- $A_{260}/A_{230}$: 3.5–4.1 |
| **SUBOPTIMAL PERFORMANCE IN DOWNSTREAM APPLICATIONS** |
| • **Carry-over of ethanol** |
| Ensure all ethanol is removed after the final wash step. Dry beads for 5–10 minutes at room temperature. |
| **CARRY-OVER OF BEADS** |
| • **Insufficient magnetic separation time** |
| Increase separation time to ensure beads are fully attracted to the magnetic pins. |