**User Guide**

# NuPure Size Selection Kit

For purification of DNA fragments

F or library preparation in next-generation sequencing

| | |
|---|---|
| **Version** | **190422** |
| **Catalog** | **K1919-SMP** |
| | **K1919-100** |

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## Product description

The NuPure Size Selection Kit is designed for tunable size selection of nucleic acid fragments in the library construction process for next generation sequencing (NGS). The magnetic beads in this kit feature a high binding capacity, slow sedimentation rate and are suspended in a special binding buffer that facilitates ratio-metric size selection of NGS input DNA and library prep adapter ligation end products.

## Contents and storage

Reagents have a one (1) year expiration date from date of purchase when stored properly.

| NuPure PCR Cleanup<br>Catalog no. | 4 preps [3]<br>K0321-SMP | 100 preps [3]<br>K0321-100 | Storage |
|---|---|---|---|
| NuPure Beads [1] | 500 ul | 10 ml | 2-8°C |
| Wash Buffer [1]<br>*Customer adds Ethanol* | 400 ul | 8 ml | 15-30°C |
| Elution Buffer [1] | 500 ul | 5 ml | 15-30°C |

**Note**. Some reagents in each kit are provided more than the amount required.

[1] NuPure Beads are also available as Catalog no. A1419 and Wash Buffer is available as Catalog no. R2300 and Elution Buffer is available as R0510.

[2] Prior to first time use, add the required amount of alcohol as indicated on the label of each bottle, then check the box and mix well.

[3] The number of preps or isolations are based on 50 ul NGS library per prep.

## Required materials not supplied

| Item | Source | Catalog no. |
|---|---|---|
| Ethanol, absolute (100%) | Fisher Scientific | BP291914 |
| Micro- and conical tubes (1-15 ml) | Various | Not applicable |
| Magnetic separator for micro-tubes | Ocean NanoTech | MMS |
| Vortex mixer | Various | Not applicable |
| Pipettors (20-1000 ul) and tips | Various | Not applicable |

## Principle

The magnetic bead technology in this kit enables ratio-metric control of DNA fragment size isolation for downstream processing.  By adjusting the volume of NuPure Beads to sample volume, one can tune the size distribution of isolated nucleic acids. After a quick binding reaction, beads are washed, air-dried and nucleic acids are eluted in a user-defined volume.

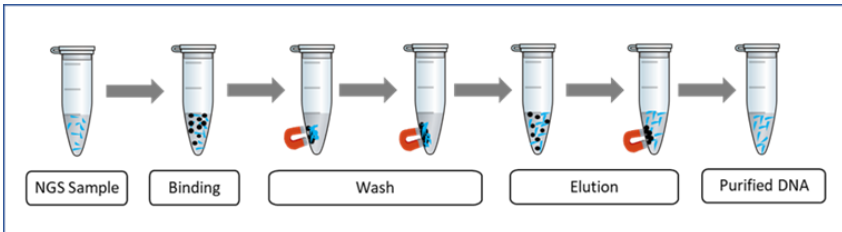


**Figure**. Illustration showing magnetic separation of DNA fragments using the NuPure Size Selection Kit.

## Procedural guidelines

- Read the USER GUIDE and make sure all the directions are followed and all recommended volumes are used as indicated.
- Prior to use, bring all kit components to room temperature.
- All vortex steps should be performed at maximum speed to ensure mixing.
- NuPure Beads should be resuspended to a homogenous suspension prior to use.

## Before you begin

**Prepare working buffers**

Prior to first time use, add ethanol (100%) as indicated on the label of each bottle(s) of Wash Buffer to obtain a working solution. Check the box on the label and mix well by inverting 10 times.

## Protocol: Isolate and purify DNA fragments from a NGS library sample

This *NuPure* Size Selection Kit can be used to generate DNA fragment libraries with a certain size range or to narrow fragment size distribution. The following protocol is for size selection of DNA fragments with a size range of 200-300 bp from 50 µl of DNA sample. By altering the volume ratios, DNA fragment libraries with other size ranges can be obtained. Please refer to the following table to guide your single sided selection of other bead-to-sample ratios if you need to modify your selection range to cover values not encompassed by our example.

**Table**. Single sided selection

| Fragments to be retained | NuPure Bead to Sample Ratio |
|---|---|
| ≥ 100 bp | 1.8x |
| ≥ 150 bp | 1.2x |
| ≥ 200 bp | 1.0x |
| ≥ 250 bp | 0.9x |
| ≥ 300 bp | 0.7x |

**Table**. Double sided selection

| Fragment range (bp) | Bead to Sample Ratio<br>1st bead selection – 2nd bead selection |
|---|---|
| 200-300 | 0.9x – 0.15x |
| 300-400 | 0.7x – 0.15x |

**1st bead selection to remove small fragments**

1. Add 45 ul (0.9x) NuPure Beads and 50 ul fragmented DNA sample to a micro tube.
2. Pulse vortex the sample or pipette up and down 10 times to bind the DNA to the beads. Then incubate the mixture at room temperature for 5 minutes.
3. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet.

4. Without removing the tube from the magnetic separator, carefully aspirate the supernatant without disturbing the pellet and transfer the supernatant to a new tube.

   ***Note***. *If you are using this kit to perform a single sided selection, discard the supernatant and while keeping Beads on the magnetic separator proceed to "Wash twice with Wash Solution" section of the protocol. For double sided selection, Do not discard the supernatant and proceed to 2nd Bead selection to remove large fragments and bind DNA.*

**2nd bead selection to remove large fragments and bind DNA**

1. To the retained supernatant, add 7.5 µl (0.15x) of NuPure Beads**.**
2. Pipet mix 10 times to bind the DNA to the beads.
3. Incubate sample mix at room temperature for 5 mins.
4. Place the tube(s) into a magnetic separator for 2 mins or until the solution clears and beads have separated from solution.
5. Without removing the tube(s) from the magnetic separator, carefully aspirate the supernatant without disturbing the pellet and discard the supernatant.

   ***Caution***. Do not discard the supernatant!

**Wash NuPure Beads with wash buffers**

5. Keep tubes on the magnetic separator. Add 200 ul of Wash Buffer and incubate for 30 seconds. Then discard all the supernatant.
6. Repeat Steps 5 for a second wash with Wash Buffer.
7. Keep the tube on the magnetic separator with the lid open. Air dry the beads for 5 minutes. Remove any visible supernatant without disturbing the beads.

   ***Caution***. *Do not over dry beads as it may result in loss of cfDNA.*

**Elute the cfDNA**

8. Remove the tube from magnetic separator and add 50 ul of Elution Buffer. Completely resuspend beads by pipetting up and down 10 times. Then incubate for 2 minutes at room temperature.
9. Place the tube on the magnetic separator for 2 minutes or until the solution clears and beads are pelleted.

10. Carefully transfer the supernatant containing the purified genomic DNA without disturbing the pellet to a clean, labeled microcentrifuge tube.

    *Note. The purified DNA is ready for immediate use. Alternatively, you can store the DNA at 4°C for up to 24 hours or -20°C for long-term storage.*

# Document History

| Version | Person | Description of Change |
| --- | --- | --- |
| 190702 | Luis Moreno | Initial draft |
| 180716 | Luis Moreno | Consultation draft – to Alice Bu |
| 190422 | Luis Moreno | Final version – approved by Luis Moreno |

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## Warranties and Disclaimers

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