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Size-Select Kit for NGS Library Preparation — Product Insert

Product # 53600

Norgen's Size-Select Kit for NGS Library Preparation provides a rapid, simple and efficient procedure for the size selection and clean-up of fragmented DNA. The resulting DNA is suitable for use in next generation sequencing library preparation. The kit selectively isolates DNA ranging in size from approximately 200-500 bp from a mixture of fragmented DNA, and removes various impurities including un-incorporated adaptors and enzymes, without the use of magnetic beads or agarose gel extraction. The kit is robust and is compatible with input volumes of up to 10 µg. The purified and size selected DNA can then be used in subsequent PCR reactions for next generation sequencing library preparation.

Norgen's Size Selection and Purification Technology

Size selection is based on first removing large DNA fragments from your mixture using the Size-Select Buffer. The small DNA, remaining in the supernatant (<400-500bp), is then transferred to Norgen's spin column. The DNA is preferentially purified from other impurities such as proteins and salts without the use of phenol, chloroform, or magnetic beads. The process involves first mixing the fragmented and adaptor-ligated DNA sample with the Size-Select Additive and Size-Select Buffer and centrifugation to remove DNA sizes >500 bp. Next, the Binding Solution is added to the supernatant and then is loaded onto a spin-column. Norgen's column binds nucleic acids in a manner that depends on ionic concentrations. Thus only the nucleic acids will bind to the column, while the contaminating proteins or nucleotides will be removed in the flowthrough. The bound nucleic acid is then washed with the provided Wash Solutions in order to remove DNA sizes of <200bp, such as smaller genomic DNA fragments, unligated adaptors and adaptor dimers. After washing, the DNA is eluted in a 30 µL volume.

The kit is designed to process 25 samples.

Specifications:

Kit Specifications	
Column Binding Capacity	10 µg of DNA
Maximum Column Loading Volume	600 µL
Size of DNA Purified	Approximately 200-400 bp
Time to Complete 10 Purifications	25 minutes
Minimum Elution Volume	30 µL

Advantages:

- Column purification – DNA is column cleaned, eliminating hazardous and labor-intensive phenol-based procedures, or costly magnetic beads.
- Selection of DNA sizes of 200bp-500bp for NGS - Efficient size separation and removal of buffers and enzymes in addition to unincorporated adaptors.
- Rapid procedure – Size-select and concentrate samples in 25 minutes.
- Provides high quality DNA fragments - The purified DNA fragments are of the highest quality and can be used for next generation sequencing library preparation.

Kit Components:

Component	Contents
Size-Select Additive	0.3 mL
Size-Select Buffer	2 x 1 mL
Binding Solution	12 mL
Wash Solution I	15 mL
Wash Solution II	6 mL
Elution Buffer	2 mL
Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
Product Insert	1

Storage Conditions and Product Stability
The **Size-Select Buffer** should be kept tightly sealed and stored upright, away from light at 4°C. All other solutions should be kept tightly sealed and stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance.

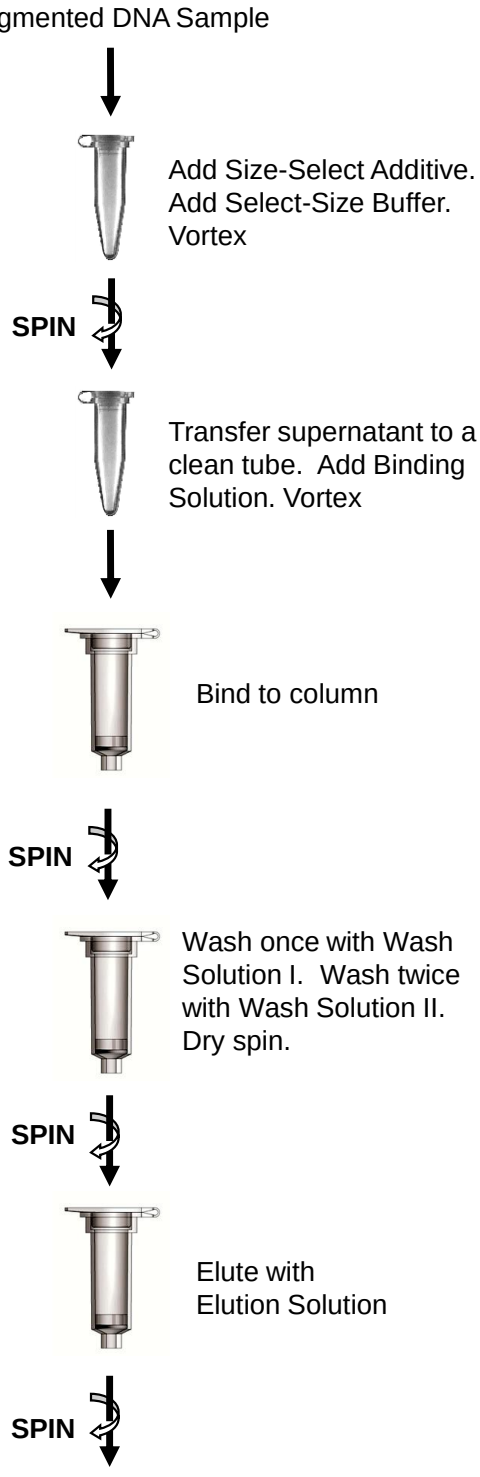
Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

The **Binding Solution** and **Wash Solution I** contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment
- Benchtop microcentrifuge
- Micropipettors
- Nuclease-free water
- 96-100% ethanol (to be added to wash buffer)

Flow Chart

Procedure for DNA size selection using Norgen's Size-Select Kit for NGS Library Preparation



Procedures
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Notes Prior to Use

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- The Size-Select Buffer can be used directly from the fridge at 4°C. Ensure that all other solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution II** by adding 23 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 29 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The maximum sample input volume that can be processed is 190 µL.

1. **Size-Select Preparation**
   a. The protocol can be used to process up to 190 µL of fragmented DNA. If the volume is less than 190 µL, add nuclease-free water to adjust the volume of the sample to 190 µL.
   b. Add 10 µL of **Size-Select Additive** and mix.
   c. Add 71 µL of **Size-Select Buffer** and mix thoroughly by vortexing for 10 seconds.
   d. Centrifuge at 10,000 x g for 15 minutes. Ensure the back of the microcentrifuge tube is oriented to the outside of the rotor. A pellet may not be visible, but this will ensure its location will be on the back wall of the tube.
   e. Carefully transfer the supernatant to a clean microcentrifuge tube (not provided). Do not disturb the DNA pellet, which will be present on the back wall of the microcentrifuge tube.

2. **Sample Binding**
   a. Add 400 µL of **Binding Solution** and mix by vortexing.
   f. Assemble a spin column with one of the provided collection tubes.
   g. Apply the mixture onto the column and centrifuge for 1 minute at 8,000 RPM.
   h. Discard the flowthrough and reassemble the column with its collection tube.

   Note: Ensure the entire solution has passed through the column into the collection tube by inspecting the column. If the entire volume has not passed, spin for an additional minute.

3. **Column Wash**
   a. Apply 500 µL of **Wash Solution I** to the column and centrifuge for 1 minute at 8,000 x g (~8,000 RPM).

   Note: Ensure the entire Wash Solution I has passed through the column into the collection tube by inspecting the column. If the entire volume has not passed, spin for an additional minute.

   b. Discard the flowthrough and reassemble the spin column with its collection tube.

c. Apply 500 µL of **Wash Solution II** to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).

Note: Ensure the entire Wash Solution II has passed through the column into the collection tube by inspecting the column. If the entire volume has not passed, spin for an additional minute.

d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Repeat steps 3c and 3d to wash the column another time with **Wash Solution II**.
f. Spin the column for 2 minutes at **14,000 x *g* (~14,000 RPM)** in order to thoroughly dry the resin. Discard the collection tube.

4. Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 30 µL of **Elution Buffer** to the column and let sit at room temperature for 1 minute.
c. Centrifuge 1 minute at **14,000 x *g* (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14 000 x g (~14,000 RPM) for 1 additional minute.

5. Storage of Purified and Size-selected DNA

The purified DNA samples should be stored at -20°C, or can be used immediately in subsequent steps of a library preparation protocol.

Related Products	Product #
Total RNA Purification Kit	17200
PCR Purification Kit	14400
10X RNA Fragmentation Buffer	53500
MiniSizer 50 bp DNA Ladder	11200

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	An alternative elution solution was used	It is recommended that the Elution Buffer supplied with this kit be used for maximum DNA recovery.
	Accurate amount of the Size-Select Additive and Size-Select Buffer were not used	Ensure that the appropriate amount of the Size-Select Additive and Size-Select Buffer is added to the sample.
	Ethanol was not added to Wash Solution II	Ensure that the correct volume of 96-100% ethanol is added to the supplied Wash Solution II prior to use.
Clogged Column	High amounts of DNA in the input	Ensure that no more than 10 µg of DNA is used as the input for each column.
	The initial centrifugation and supernatant recovery was not performed	Ensure that the initial 15 min centrifugation after mixing the sample and the Size-Select Additive and Size-Select Buffer was performed and the supernatant was transferred to a clean tube
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.
DNA does not perform well in downstream applications	DNA was not washed with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed for the specified wash times with the Wash Solutions. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

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