

## NGS Normalization 96-Well Kit

## Product Insert

Product# 61900

Norgen's NGS Normalization 96-Well Kit allows for high-throughput NGS library PCR amplicon clean-up and normalization of NGS library PCR product concentration (~5±1 ng/μL). The clean-up removes all PCR by-products including primers, dimers, enzymes, and unincorporated nucleotides during the library preparation PCR step. Purification is based on 96-well column chromatography using Norgen's proprietary resin as the separation matrix. The amount of Norgen's matrix in each 96 well is optimized to have a limited binding capacity, thus each well can elute an equal concentration of PCR product. The process involves first adding 3 volumes of Buffer SK to the PCR product, and the mixture is then loaded onto the 96-Well Normalization Plate. The bound PCR amplicon is then washed with the provided Wash Solution A in order to remove any remaining impurities, and the purified PCR amplicon is eluted with Elution Buffer B. The purified and normalized library PCR amplicon can then be used in NGS workflow and other sequencing applications.

### Kit Components:

Component	Product # 61900 (192 Preps)
Buffer SK	30 mL
Wash Solution A	2x 38 mL
Elution Buffer B	30 mL
Adhesive Tape	1
96-Well Normalization Plate	2
96-Well Collection Plate	2
96-Well Elution Plate	2
Product Insert	1

### Advantages

- Remove primer dimers left during NGS library PCR
- Simultaneously clean-up and normalize libraries
- Fast, high-throughput and easy processing using centrifuge
- Sufficient elution volume (100 μL) for repeat or future assays
- Non-magnetic bead purification

### Customer-Supplied Reagents and Equipment

- Centrifuge with rotor for 96-well plate assembly (such as Eppendorf 5810R, Thermo Fisher IEC Centra CL3 series or Beckman GS-15R)
- Micropipettors and multichannel pipettes
- DNase-free, aerosol barrier pipette tips
- 96-100% Ethanol

### Storage Conditions

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

### General 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.

**Buffer SK** contains 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. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at [www.norgenbiotek.com](http://www.norgenbiotek.com).

**CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.**

### Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

## Protocol

### 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.
- Prepare a working concentration of **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottles containing concentrated **Wash Solution A**. This will give a final volume of 128 mL. The labels on the bottles have a box that can be checked to indicate that ethanol has been added.

1. Add 3 volumes of **Buffer SK** directly to each well containing the library PCR extension product. (For example: Add 60  $\mu$ L of **Buffer SK** to 20  $\mu$ L of library PCR product). Mix by pipetting up and down or seal the plate with Adhesive Tape vortex and briefly centrifuge the plate.
2. Place the 96-Well Normalization Plate on top of a provided 96-Well Collection Plate.
3. Add the mixture into the wells of the 96-Well Normalization Plate.
4. Centrifuge the assembly at maximum speed or 3,200 x g (~4,000 RPM) for 2 minutes.
5. Discard the flowthrough. Reassemble the 96-Well Normalization Plate and the 96-Well Collection Plate.

**Note:** Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

6. Apply 400  $\mu$ L of **Wash Solution A** to each well of the 96-Well Normalization Plate. Centrifuge the assembly at maximum speed or 3,200 x g (~4,000 RPM) for 2 minutes.

**Note:** Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

7. Discard the flowthrough. Reassemble the 96-Well Normalization Plate and the 96-Well Collection Plate.
8. Repeat steps 6 and 7 to wash the plate a second time.
9. Centrifuge the assembly at maximum speed or 3,200 x g (~4,000 RPM) for 15 minutes in order to completely dry the plate.
10. Gently pat the bottom of the 96-Well Normalization Plate on paper towels.
11. Stack the 96-Well Normalization Plate and the 96-Well Elution Plate (provided).
12. Add 100  $\mu$ L of **Elution Buffer B** to each well of the 96-Well Plate.
13. Centrifuge the assembly at 200 x g (~1,000 RPM) for 1 minute followed by 3,200 x g (~4,000 RPM) for 4 minutes.

### Storage of DNA

Use the provided adhesive tape to seal the Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

## Troubleshooting Guide

Possible Cause	Solution and Explanation
Irregular PCR amplicon concentration	Ensure that 3 volume of Buffer SK is added to and mixed well with the library PCR product. The ratio between library PCR product and Buffer SK is important and has to be 1:3.
Ethanol carryover	Ensure that the dry spin under Step 8 is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

Related Products	Product #
Sequencing Reaction Clean-Up Kit	34500, 34400
PCR Purification Kit	14400, 24800, 45700
PCR and Sequencing Reaction Clean-Up Kit (Magnetic Bead System)	60200

### Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's NGS Normalization 96-Well Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please 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 or call one of the NORGEN local distributors ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6  
Phone: (905) 227-8848  
Fax: (905) 227-1061  
Toll Free in North America: 1-866-667-4362