NORGEN BIOTEK CORPORATION
Advion Interchim scientific
3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Oligo Clean-Up and Concentration Kit

Product Insert

Product # 34100

Norgen's Oligo Clean-Up and Concentration Kit provides a rapid, simple and efficient procedure for the purification and concentration of up to 10 µg of oligonucleotides. The kit is used for the purification of synthesized oligonucleotides, as well as the clean-up of oligonucleotides from various upstream enzymatic reactions such as ligation, Poly(A) tailing and end-labeling. Single or double stranded DNA or RNA oligonucleotides larger than 10 bp can be purified with the kit, therefore the kit is not recommended for the removal of PCR primers. The kit purifies oligonucleotides from other reaction components including proteins, buffers and nucleotides without the use of phenol, chloroform, alcohol precipitation or urea PAGE extraction. The kit provides a high quality product with up to 90% recovery. The purified oligonucleotides can be used in a number of downstream applications including end-point or quantitative reverse transcription PCR, Northern blotting, *in situ* hybridization, and RNAi studies.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The DNA oligonucleotide or RNA oligonucleotide is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first mixing the sample containing the oligonucleotide with Buffer RL (please see the flow chart on page 3). Isopropanol is then added and the mixture is loaded onto a spin-column. Norgen's resin 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 three times with the provided Wash Solution A in order to remove any remaining impurities. The purified oligonucleotide is then eluted with Elution Solution A. The purified oligonucleotide is of the highest integrity, and can be used in a number of downstream applications.

The kit is designed to process 50 oligonucleotide samples.

Specifications:

Kit Specifications	
Column Binding Capacity	10 µg for DNA or RNA
Maximum Column Loading Volume	600 µL
Size of DNA/RNA Purified	> 10 nt, single stranded or double stranded
Maximum Amount of Starting Material	10 µg of DNA or RNA
Time to Complete 10 Purifications	15 minutes
Minimum Elution Volume	20 µL

Advantages:

- Process all oligonucleotides – The kit can clean and concentrate both DNA and RNA oligonucleotides that are single or double stranded.
- Complete column purification - The RNA or DNA oligonucleotides are column cleaned and/or concentrated, eliminating labor-intensive PAGE-based purification.
- Cleans enzymatic reaction mixtures - Efficient removal of buffers and enzymes in addition to dNTPs.
- Rapid procedure – Clean and concentrate 10 oligonucleotide samples in 15 minutes.
- Provides high quality oligonucleotides - The purified oligonucleotides are of the highest quality and can be used in a number of downstream applications.

Kit Components:

Component	Contents
Buffer RL	30 mL
Wash Solution A	20 mL
Elution Solution A	6 mL
Micro Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

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

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*.

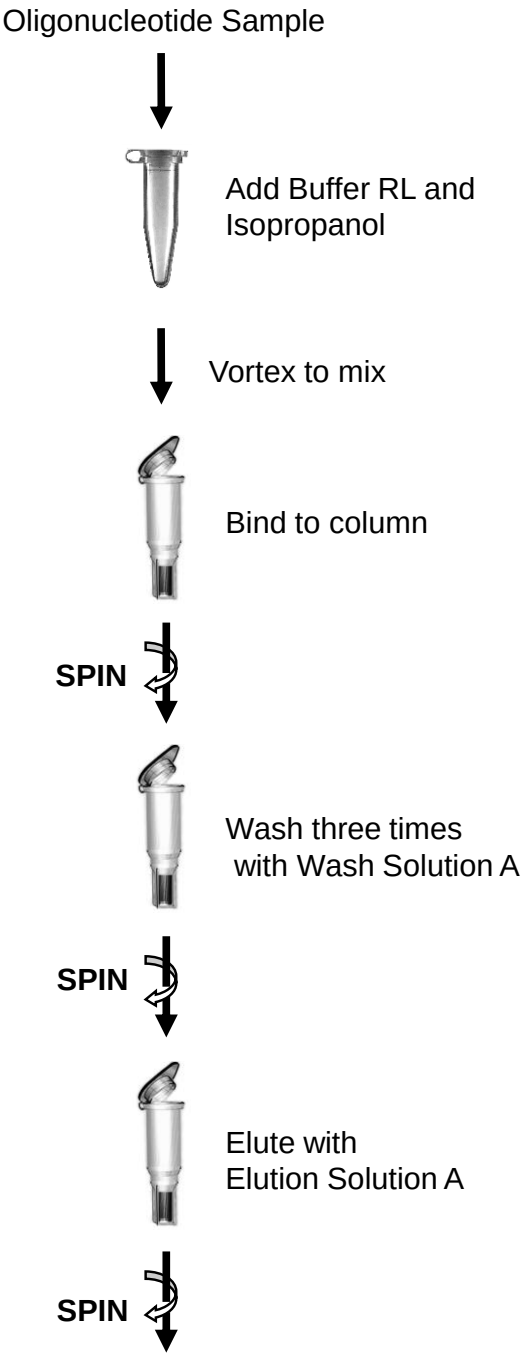
Customer-Supplied Reagents and Equipment
- Benchtop microcentrifuge
- Micropipettors
- Nuclease-free water
- β-mercaptoethanol
- Isopropanol
- 96-100% ethanol

Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

Flow Chart

Procedure for oligonucleotide purification using Norgen's Oligo Clean-Up and Concentration Kit



Purified Oligonucleotide Sample

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.
- Ensure that all solutions are at room temperature prior to use.
- Prepare an appropriate amount of **Buffer RL** by adding 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of Buffer RL required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- Prepare a working concentration of the **Wash Solution A** by adding 80 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 100 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is recommended that no more than 10 µg of oligonucleotides to be used per column.
- It is important to work quickly during this procedure when cleaning RNA oligonucleotides.
- The maximum sample input volume that can be processed is 200 µL.

1. Sample Preparation

a. Adjust the volume of the oligonucleotide sample to 50 µL by adding nuclease-free water. It is recommended that no more than 10 µg of oligonucleotides be used for each column.

Note: For the concentration of oligonucleotides, sample volumes up to 200 µL may be processed.

b. Add 150 µL of **Buffer RL** to the oligonucleotide sample.

c. Add 300 µL of isopropanol (provided by the user) to the mixture from step **1b.** Mix by vortexing for 10 seconds.

Note: When processing larger sample volumes ensure that proportional amounts of the Buffer RL and Isopropanol are added. (ie. The ratio of Sample : Buffer RL: Isopropanol must be maintained at 1 : 3 : 6).

2. Binding to Column

a. Assemble a column with one of the provided collection tubes.

b. Apply the up to 600 µL of the oligonucleotide sample with the isopropanol (from **Step 1c**) onto the column and centrifuge for 1 minute at **14,000 x *g* (~14,000 RPM)**.

c. Discard the flowthrough. Reassemble the spin column with its collection tube.

d. Repeat steps 2b and 2c as required to bind the entire sample to the column.

3. Column Wash

a. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 1 minute at **14,000 x *g* (~14,000 RPM)**.

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

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps **3a** and **3b** to wash the column a second time.
d. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
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. Oligonucleotide Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 µL of **Elution Solution A** to the column.

Note: For higher concentrations of oligonucleotides, a lower elution volume may be used. A minimum volume of 20 µL is recommended

c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 2 minutes 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 Oligonucleotides

The purified DNA oligonucleotide sample may be stored at –20°C. Purified RNA oligonucleotide samples should be stored at –70°C.

Frequently Asked Questions

1. What If a variable speed centrifuge is not available?

- A fixed speed centrifuge can be used, however reduced yields may be observed.

2. What will happen if my centrifugation speed varied from the recommended speed?

- This may lead to reduction in the oligonucleotide yields.

3. Can I process a different oligonucleotide volume?

- Yes, you can. All the solutions included in the sample preparation step of this kit are in a linear relationship to the volume of oligonucleotide sample processed. Make sure that you do not deviate from the ratio specified in the product manual. The solutions are optimized per 50 µL of oligonucleotide sample.

4. What If I added more or less from the specified reagents' volume?

- Adding less volume may reduce your oligonucleotide yields. Adding more may not affect the oligonucleotide yields EXCEPT if more Elution Solution A was added. Eluting oligonucleotide in more Elution Solution will result in diluting your yield.

5. What If I forgot to do a dry spin after my third wash?

- Your oligonucleotide elution will be contaminated with the Wash Solution A. This may dilute the oligonucleotide yield in your elution. Also, it may interfere with your down stream applications.

6. What If the recovery of the oligonucleotide is poor?

- Make sure that isopropanol was added in the sample preparation step and ethanol was added to the Wash Solution A. Also, it is recommended that the Elution Buffer supplied with this kit be used for maximum RNA recovery.

7. Why my oligonucleotide does not perform well in downstream applications?

- Traces of salt from the binding step may remain in the sample if the column is not washed three times with the Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column. 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. If a different Elution Solution was used other than the one provided in the kit, the solution should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your elution buffer with the intended use.

Related Products	Product #
RNA Clean-Up and Concentration Kit	23600
PCR Purification Kit	14400
CleanAll DNA/RNA Clean-Up and Concentration Kit	23800
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.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

Advion Interchim scientific

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