

ACQUITY UPLC Oligonucleotide C₁₈ Columns

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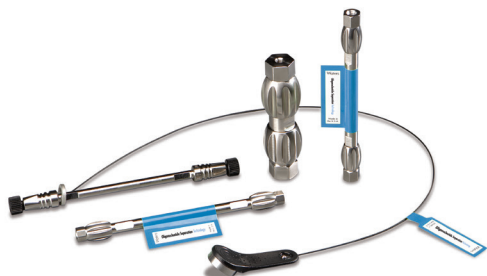
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I. INTRODUCTION

Thank you for choosing an ACQUITY UPLC® Oligonucleotide C₁₈ Column designed specifically for use on Waters ACQUITY UPLC System. The separation of detritylated oligonucleotides on Waters second generation of hybrid-silica BEH Technology™ particles are based on the well established method on ion-pair, reversed-phase chromatography. ACQUITY UPLC Oligonucleotide C₁₈ Columns are available in several configurations to address different application needs. Your ACQUITY UPLC Oligonucleotide C₁₈ packing material is manufactured in a cGMP, ISO 9002 certified plant using ultra pure reagents. Each batch of ACQUITY UPLC Oligonucleotide C₁₈ material is chromatographically tested with acidic, basic, and neutral analytes and the results are held to narrow specification ranges to assure excellent, reproducible performance. In addition, every column is individually tested and the associated Performance Test Chromatogram and Certificate of Acceptance information is available through the attached eCord Intelligent Chip Technology.

Note: Optimum performance of ACQUITY UPLC Oligonucleotide C₁₈ Columns is best assured using an appropriately configured Waters ACQUITY UPLC System (e.g., See Section II, g). Consequently, use of ACQUITY UPLC Oligonucleotide C₁₈ Columns on conventional HPLC systems is not recommended.



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I. GETTING STARTED

Each ACQUITY UPLC Oligonucleotide C₁₈ Column comes with Certificate of Analysis and a Performance Test Chromatogram embedded within the eCord Intelligent Chip. The Certificate of Analysis is specific to the batch of packing material contained in the ACQUITY UPLC Oligonucleotide C₁₈ Column and includes the gel batch number, analysis of unbonded particles, analysis of bonded particles, and chromatographic results and conditions. The Performance Test Chromatogram is specific to each individual column and contains such information as gel batch number, column serial number, USP plate count, USP tailing factor, capacity factor, and chromatographic conditions. These data should be stored for future reference.

a. Column Connectors

The ACQUITY UPLC System utilizes tubing and gold plated compression screws that have been designed to meet stringent tolerance levels and minimize extra column volumes. Optimized column inlet tubing (p/n: [430001084](#)) is supplied with the ACQUITY UPLC System. The inject valve end of the tubing is clearly marked with a blue shrink tube marker. Insert the opposite end of the tubing into the ACQUITY UPLC Column and tighten the compression fitting using two 5/16-inch wrenches. For information on the correct column outlet tubing, please refer to the relevant detector section in the ACQUITY UPLC System Operator's Guide (p/n: [71500082502](#)).

b. Column Installation

1. Purge the pumping system of any buffer-containing mobile phases and connect the inlet end of the column to the injector outlet.
2. Flush column with 100% organic mobile phase (acetonitrile with TEAA or methanol for TEA-HFIP ion-pairing method) by setting the pump flow rate to 0.1 mL/min and increase the flow rate to 0.5 mL/min over 5 minutes.
3. When the mobile phase is flowing freely from the column outlet, stop the flow and attach the column outlet to the detector. This prevents entry of air into the detection system and gives more rapid baseline equilibration.
4. Gradually increase the flow rate as described in step 2.
5. Once a steady backpressure and baseline at 260 nm have been achieved, proceed to the next section.

c. Column Equilibration

ACQUITY UPLC Oligonucleotide C₁₈ Columns are shipped in 100% acetonitrile. It is important to ensure mobile phase compatibility before changing to a different mobile phase system. Equilibrate the column with a minimum of 10 column volumes of the mobile phase to be used for the oligonucleotide separation.

Note: When mobile phase additives are present in low concentrations (e.g., TEAA or TEA-HFIP ion-pairing reagents), 100 to 200 column volumes may be required for complete ACQUITY UPLC Oligonucleotide C₁₈ Column equilibration.

Table 1. Empty column volumes in mL (multiply by 10 for flush solvent volumes).

Column length (mm)	Internal diameter 2.1 mm
50	0.2
100	0.4
150	0.5

d. eCord Installation

The eCord button should be attached to the side of the column heater module. The eCord button is magnetized and does not require specific orientation.

e. Initial Column Efficiency Determination

1. Perform an efficiency test on the column before using it. Waters recommends using a suitable solute mixture, as found in the "Performance Test Chromatogram", to analyze the column upon receipt.
2. Determine the number of theoretical plates (N) and use this value for periodic comparisons.
3. Repeat the test at predetermined intervals to track column performance over time. Slight variations may be obtained on two different UPLC Systems due to the quality of the connections, operating environment, system electronics, reagent quality, column condition, and operator technique.

ACQUITY Oligonucleotide C₁₈ Column column performance can be tested with the MassPREP™ Oligonucleotide Standard (p/n: [186004135](#)), a quality controlled synthetic oligonucleotide sample consisting of 15, 20, 25, 30, and 35 mer deoxythymidine. Approximately 0.1 nmol of each oligonucleotide was injected onto the ACQUITY UPLC Oligonucleotide C₁₈ Column. Refer to p/n: [715001677](#) for more information on sample prep for the MassPREP Oligonucleotide Standard. Smaller peaks eluting prior to the main peaks are failure, by-product sequences from the synthesis.

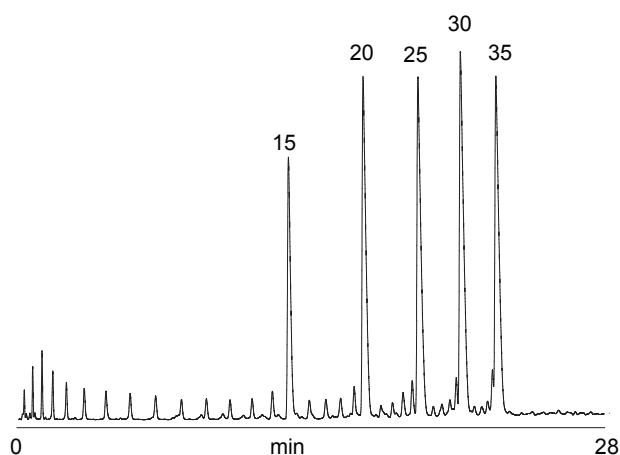


Figure 1. Separation using the MassPREP Oligonucleotide Standard on ACQUITY Oligonucleotide C₁₈.

UPLC system:	Waters ACQUITY UPLC System with installed 425 µL mixer (see Section II, g), PDA Detector with standard UV cell
Sample injected:	Approximately 0.1 nmol of MassPREP Oligonucleotide Standard (p/n: 186004135) diluted in 0.1 M TEAA
Column:	Waters ACQUITY UPLC Oligonucleotide C ₁₈ Column, 1.7 µm (2.1 x 50 mm)
Mobile phases:	A: 0.1 M TEAA B: Acetonitrile/0.1 M TEAA, 20/80, v/v
Flow rate:	0.2 mL/min
Column temp.:	60 °C
Gradient delay:	0.45 mL
Gradient:	40 to 59.5% B in 26 minutes (8–11.9% acetonitrile, 0.15% acetonitrile per minute)
Detection:	260 nm, 5 scans per second

II. COLUMN USE

To ensure the continued high performance of your ACQUITY UPLC Oligonucleotide C₁₈ Columns, follow these guidelines:

a. Sample Preparation

1. Dissolve the detritylated synthetic oligonucleotide sample in mobile phase A (e.g., 0.1 M TEAA). For example, a 0.05 - 0.2 µmole scale synthesis can be prepared in 0.1 mL of 0.1 M TEAA. Proportionately larger or smaller volumes of 0.1 M TEAA is required when dissolving samples from different scale syntheses. Due to the nature of gradient separations, relatively large volumes of sample (in low organic strength eluent) can be injected and concentrated onto the head of the column before beginning the gradient elution program.
2. Samples must be completely in solution and free of particulates. Remove all particles from the sample (Controlled Pore Glass Synthesis Support, etc.), which may block the inlet column frit, increase the operating pressure, and shorten the column life time. Sample contamination with high concentration of salts and/or detergents may also interfere with analysis.
3. To remove particulates the sample may be filtered with a 0.2 µm membrane. Be sure that the selected membrane is compatible and does not dissolve with the selected mobile phase diluent. Contact the membrane manufacturer with solvent compatibility questions. An alternative method of particulate removal involves centrifugation for 20 minutes at 8,000 rpm, followed by the transfer of the supernatant liquid to an appropriate vial.

b. Recommended Mobile Phases

The most common ion-pair mobile phase for synthetic oligonucleotide separations is based on triethylammonium acetate (TEAA). This mobile phase can be prepared by titrating glacial acetic acid aqueous solution with triethylamine (TEA).

Note: To maximize column life, it is ESSENTIAL that all prepared oligonucleotide mobile phases be filtered through a solvent compatible, 0.2 µm membrane and contained in bottles that are clean and particulate free.

TEAA

1 L of 0.1 M TEAA may be prepared as follows:

1. Perform work in a hood.
2. Add 5.6 mL of glacial acetic acid into 950 mL of water and mix well.
3. Slowly add 13.86 mL of TEA.
4. The pH should be adjusted to pH 7 +/- 0.5 by careful addition of acetic acid.
5. Adjust final volume to 1 L with water.

Alternatively, premixed TEAA can be used [e.g., Sigma 1 M TEAA (p/n: 90357)]. Mix 100 mL with 900 mL of water to prepare 1 L of 0.1 M TEAA mobile phase.

Alternative ion-pairing reagents are recommended for improved separation of phosphorothioates or when performing LC-MS analyses. An ion-pairing mobile phase based on triethylamine (TEA) and hexafluoroisopropanol (HFIP) as the buffering acid produces an efficient eluent system for improved separations involving these application types.

As indicated below, two ion-pairing systems are useful.

For routine detritylated oligonucleotide applications, aqueous buffer consisting of 8.6 mM TEA and 100 mM HFIP is effective. For applications such as those involving the separation of G-rich oligonucleotides, it is advisable to use aqueous buffer consisting of 15 mM TEA and 400 mM HFIP (pH 7.9).

TEA-HFIP System 1

1 L of 8.6 mM TEA/100 mM HFIP is prepared as follows:

1. Perform work in a hood.
2. Add 10.4 mL of HFIP (16.8 g) into 988.4 g of water and mix well.
3. Slowly add 1.2 mL of TEA.
4. The pH is approximately 8.3 +/- 0.1.

TEA-HFIP System 2

1 L of 15 mM TEA/400 mM HFIP is prepared as follows:

1. Perform work in a hood.
2. Add 41.56 mL (67.17 g) of HFIP into 956.36 g of water and mix well.
3. Slowly add 2.08 mL (1.52 g) of TEA.
4. The pH of final buffer is approximately 7.9 +/- 0.1.

c. Recommended Injector Wash Solvents

Between analyses, the ACQUITY UPLC System injector and seals can and should be washed with two separate solvents. A 90% water/10% acetonitrile mixture is the recommended strong solvent injector wash solution for the TEAA ion-pairing based method.

A 90% water/10% methanol mixture is the recommended strong solvent injector wash solution for the TEA-HFIP based method.

0.20 µm membrane filtered, LC grade water is the recommended weak wash solvent solution for all ACQUITY Oligonucleotide Separation Methods.

Note: Do not use oligonucleotide separation mobile phases A and B for the respective weak and strong injector wash solvents especially with TEA-HFIP ion pairing systems due to seal incompatibility issues with HFIP.

d. pH Range

The recommended operating pH range for ACQUITY UPLC Oligonucleotide C₁₈ Columns is 1 to 12.

e. Pressure

ACQUITY UPLC Oligonucleotide C₁₈ Columns can tolerate pressures of up to 15,000 psi (1034 bar or 103 Mpa).

f. Temperature

Temperatures between 20 °C–90 °C are recommended for operating ACQUITY UPLC Oligonucleotide C₁₈ Columns in order to enhance selectivity, lower solvent viscosity, and increase mass transfer rates.

Note: Operating at elevated pH, temperature, and/or pressure may potentially result in shortened column life.

g. ACQUITY UPLC Mixer Options

The standard Waters ACQUITY UPLC System is equipped with 50 µL in-line mobile phase mixer. For demanding biopolymer separations (e.g., peptide mapping), use of a shallow gradients (e.g., 0.15% mobile phase B change per minute) is required. In these situations, it is recommended that the organic solvent concentration in mobile phase B be reduced by “premixing” with a measured amount of mobile phase A (e.g., mobile phase A= 0.1 M TEAA and mobile phase B= acetonitrile/0.1 M TEAA, 20/80, v/v).

Use of a 425 µL mixer (p/n: [205000403](#)) specifically designed for shallow UPLC gradient separations is recommended when the solvent premixing technique (detailed above) is not used and when mobile phase B contains either 100% acetonitrile (for TEAA ion-pairing method) or 100% methanol (for TEA-HFIP ion-pairing method).

In addition, the Solvent Deliver System Outlet Tube Assembly (p/n: [430001486](#)) is required for 425 μ L mixer installation onto a standard ACQUITY UPLC System.

Note: The 425 μ L mixer introduces an additional delay volume to gradient separations. For ultra-fast oligonucleotide analyses, the smaller 50 μ L mixer should be used with the described premixed mobile phase technique.

h. Flow Rate

The recommended flow rate for oligonucleotide separations performed on a 2.1 x 50 mm ACQUITY UPLC Oligonucleotide C₁₈ Column is 0.2 mL/minute. When faster flow rates are desired for separations, use of the 425 μ L mixer with installed Outlet Tubing Assembly is recommended.

III. COLUMN CLEANING, REGENERATING AND STORAGE

a. Cleaning and Regeneration

Changes in peak shape, peak splitting, shoulders on the peak, shifts in retention, change in resolution or increasing backpressure may indicate contamination of the column. Flushing with approximately 20 column volumes of 0.2 μ m membrane filtered, neat organic solvent (e.g., acetonitrile with the TEAA method of methanol with the TEA/HFIP protocol) is usually sufficient to remove the contaminant. If the neat organic solvent flushing procedure does not solve the problem, purge the column with 20 column volumes of oligonucleotide mobile phase A followed by 20 column volumes of either 7 M guanidine hydrochloride or 7 M urea. Be sure to flush column with an additional 20 column volumes of 0.2 μ m membrane filtered, LC grade water prior to reuse of oligonucleotide mobile phases. If the column performance is poor after regenerating and cleaning, call your local Waters office for additional support.

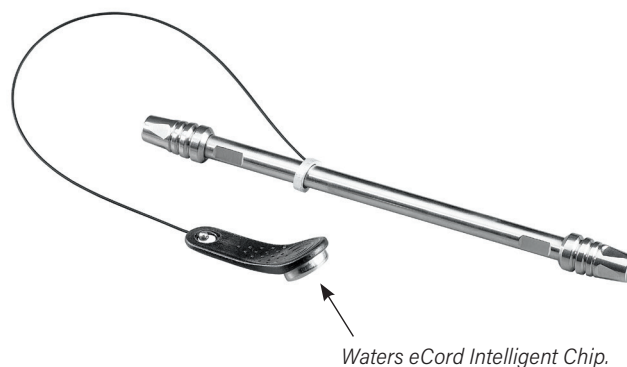
b. Storage

For periods longer than four days at room temperature, store the column in 100% acetonitrile. Immediately after use at elevated temperature and/or pH, store column in 100% acetonitrile for the best column lifetime. Do not store column in highly aqueous (<20% organic) mobile phase, as this may promote bacterial growth. Completely seal column to avoid evaporation and drying out of the packed bed.

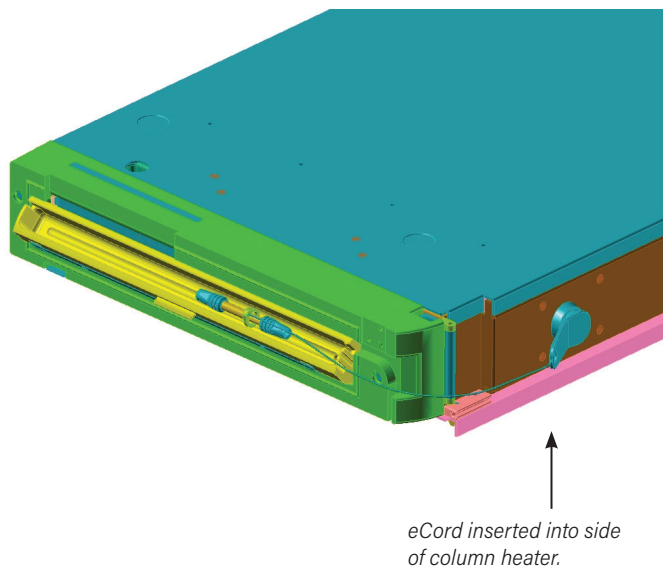
IV. eCORD INTELLIGENT CHIP TECHNOLOGY

a. Introduction

The eCord Intelligent Chip is a technology that will provide the history of a column's performance throughout its lifetime. The eCord is permanently attached to the column to assure that the column's performance history is maintained in the event that the column is moved from one instrument to another.



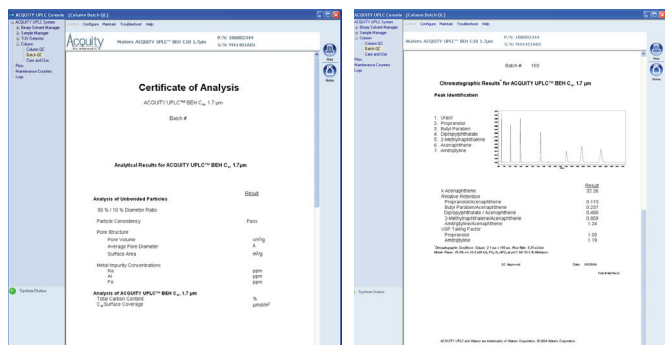
At the time of manufacture, tracking and quality control information will be downloaded to the eCord. Storing this information on the chip will eliminate the need for a paper Certificate of Analysis. Once the user installs the column, the software will automatically download key parameters into a column history file stored on the chip. The eCord provides a solution to easily track the history of column usage.



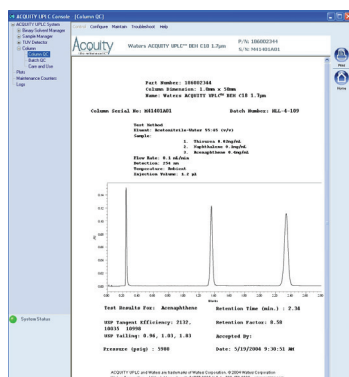
b. Installation

Install the column into the column heater. Plug the eCord into the side of the column heater. Once the eCord is inserted into the column heater the identification and overall column usage information will be available in Empower® and MassLynx® software allowing the user to access column information on their desktop.

c. Manufacturing Information

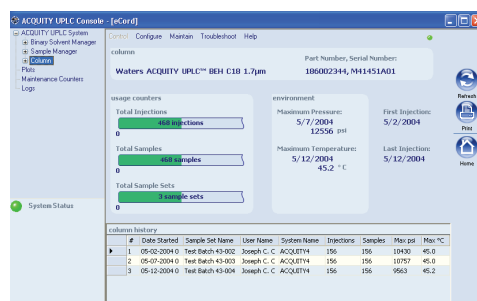


The eCord Intelligent Chip provides the user with an overview of the bulk material QC test results.



The eCord Intelligent Chip provides the user with QC test conditions and results on the column run by the manufacturer. The information includes mobile phases, running conditions and analytes used to test the columns. In addition the QC results and acceptance is placed onto the column.

d. Customer Use Information



The eCord Intelligent Chip will automatically capture column use data. The top of the screen identifies the column including chemistry type, column dimensions and serial number. The overall column usage information includes the total number of samples, total number of injections, total sample sets, date of first injection, date of last injection, maximum pressure, and temperature. The information also details the column history by sample set including date started, sample set name, user name, system name, number of injections in the sample set, of samples in the sample set, maximum pressure and temperature in the sample set, and if the column met basic system suitability requirements.

V. ADDITIONAL INFORMATION

a. Band Spreading Minimization

Waters ACQUITY UPLC System is designed to have a minimal post column band spreading. If desirable, the mass spectrometer can be connected either directly or in series with a UV (PDA) detector. The connecting tubing internal diameter should be 100 µm or less in order to preserve the achieved separation. Length of the tubing should also be kept to a minimum.

Detritylated synthetic oligonucleotide separations are almost exclusively performed using gradient elution techniques. As such, the effect of pre-column sample band broadening can be minimized by allowing the sample to bind to the column before beginning the actual separation gradient. However, proper connection from the ACQUITY UPLC Oligonucleotide C₁₈ Column outlet to the detector is critical in order to minimize the deleterious effect of p-column sample band spreading. Use of appropriate internal diameter tubing (e.g., 0.005 inch PEEK™ tubing for UV detector applications) is recommended.

Table 2. Ordering information.

Description	Particle Size	Dimension	P/N
ACQUITY UPLC Oligonucleotide C ₁₈ , 135Å Column*	1.7 µm	2.1 x 50 mm	186003949
ACQUITY UPLC Oligonucleotide C ₁₈ , 135Å Column*	1.7 µm	2.1 x 100 mm	186003950
ACQUITY UPLC Oligonucleotide C ₁₈ , Custom Column*	-	-	186003951
MassPREP Oligonucleotide Standard	-	-	186004135

*Optimum performance of ACQUITY UPLC Oligonucleotide C₁₈ Columns is best assured using an appropriately configured Waters ACQUITY UPLC System. Consequently, use of ACQUITY UPLC Oligonucleotide C₁₈ Columns on conventional HPLC systems is not recommended.

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