# MASSPREP OLIGONUCLEOTIDE SEPARATION TECHNOLOGY STANDARD

## I. INTRODUCTION

The pre-packaged MassPREP™ Oligonucleotide Separation Technology (OST) Standard is designed for verification of HPLC/UPLC® instrument and column performance for analysis of synthetic oligonucleotides. Approximately equimolar amounts of 15, 20, 25, 30 and 35 nucleotide (nt) long oligodeoxythymidines are lyophilized and packaged in 1.5 ml LC vials. These vials are vacuum-sealed in foil pouches to reduce degradation that can occur by excessive exposure to light and air. Approximately 1 nmole of each oligonucleotide is present in the vial.

## II. RECOMMENDED USAGE

Waters MassPREP OST standard is intended for testing the column and HPLC/UPLC systems separation performance. For selection of Waters XBridge™ OST C18, 2.5 µm HPLC or ACQUITY UPLC® OST C18, 1.7 um UPLC column, see document 720002008EN on waters.com/library. Both columns and LC system performance can be tested using this dedicated MassPREP OST standard. The most common problems in oligonucleotide analysis that can be detected using OST standard are listed in the Table 1.

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*Table 1: System Troubleshooting using MassPREP OST Standard*

| Chromatogram Appearance | Potential problem |
|---|---|
| Peaks elute outside of the expected retention time window | Incorrectly prepared mobile phase, aged mobile phase, incorrect column temperature setting; excessive gradient delay (method transfer between different LC systems). |
| Tailing peaks | Poor tubing connections (especially between column and detector); column bed deterioration, column contamination. |
| Inconsistent peak width, split peaks | Inadequate gradient mixing, incompatible sample solvent or weak wash (purge solvent for Alliance® HT). |
| Lost resolution | Aged or contaminated column. (Has column backpressure changed?) |

## III. PREPARATION PROCEDURE

The following procedure is provided as a general guideline for MassPREP OST standard reconstitution. The described method only serves as a starting point. Depending on the specific application, one may consider using other solvents and/or dilutions. Recommended chemicals to prepare sample diluent and LC mobile phase are following: Acetic Acid (2M):Triethylamine (2M), Fluka, P/N 09748; 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), Fluka, P/N 52512; triethylamine (TEA), Sigma, P/N 417283.

1. Prepare 100 mM of triethylammonium acetate (TEAA) by diluting a 2M:2M stock solution of Acetic Acid:Triethylamine 20 fold in deionized or HPLC grade water.
2. Add 0.5 mL of 100 mM TEAA in MassPREP OST standard vial. The final concentration is ~2 pmole/µL for each oligonucleotide. Vortex the vial briefly to dissolve and homogenize the sample.
3. For MassPREP OST standard analysis in UPLC mode, prepare the following mobile phases:
   a. Mobile phase A is 15 mM TEA, 400 mM HFIP in water. Add 8.31 mL (13.44 g) of HFIP into 191.3 g of water, add 416 µL of TEA; the final volume is 200 mL, the buffer pH is ~7.9.
   b. Mobile phase B is 50% A, 50% MeOH. Prepare TEA-HFIP buffer as described above, and add 158.2 g of MeOH (200 mL).
   c. The LC solvents are highly volatile; the mobile phase should be used only 24 hours only before making a fresh one. Keep the mobile phase containers sealed to minimize the buffer evaporation.
4. Prime the ACQUITY UPLC system and connect a 2.1 x 50 mm ACQUITY UPLC OST C18, 1.7 µm column (P/N 186003949). Setup flow rate at 0.2 mL/min, and column temperature at 60 °C. Equilibrate with initial mobile phase conditions (38% B) for ~20 minutes.
5. Inject 10 µL of the MassPREP OST standard. Run the gradient from 38 to 50% B in 12 minutes. The example of chromatogram is shown in Figure 1.
6. The MassPREP OST sample can also be used to troubleshoot XBridge OST C18, 2.5 µm columns configured to a HPLC system. The resolution is not expected to match the performance obtained with a ACQUITY UPLC OST C18, 1.7 µm column and UPLC System.
7. The alternative conditions for HPLC (UPLC) oligonucleotide analysis use TEAA ion-pairing system:
   a. Mobile phase A is 100 mM TEAA (measure 190 g of water, add 10 mL of 2M:2M Acetic Acid:Triethylamine Stock Solution. The pH adjustment is not necessary.
   b. Mobile phase B: 20% acetonitrile, 80% TEAA. Prepare 200 mL of TEAA as above, and add 50 mL (39.3 g) of acetonitrile.
   c. The most useful analytical column for HPLC is a 2.1 x 50 mm, XBridge OST C18, 2.5 µm column (P/N 186003952). Flow rate is set to 0.2 mL/min, column temperature to 60 °C. Gradient is 40-60% B in 25 minutes. Approximately 20-40 µL of the MassPREP OST sample is typically injected on column (80 pmole per peak). Example of chromatogram is not shown.

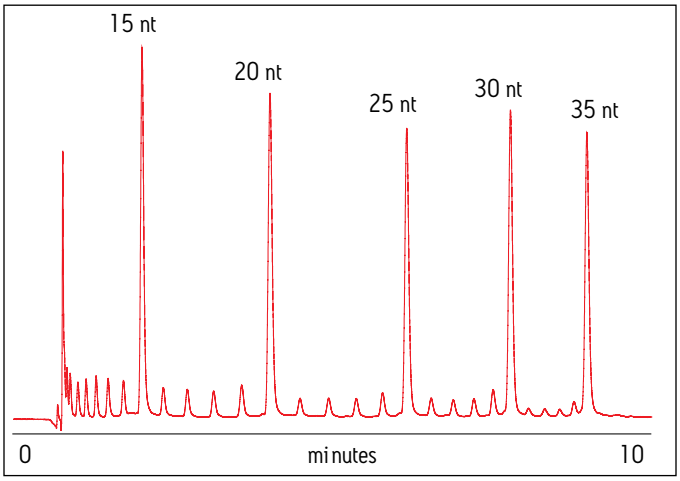


*Figure 1: ACQUITY UPLC analysis of MassPREP OST standard on an ACQUITY UPLC OST C18, 1.7 µm column. The main components are labeled. Small peaks eluting between labeled oligonucleotides are N-1, N-2, etc. failure sequences generated during the oligonucleotide syntheses. The ACQUITY UPLC system is equipped with 50 µL standard mixer and PDA detector (260 nm).*

## IV. STORAGE AND STABILITY

It is recommended that the MassPREP OST Standard remain in the foil pouch (in the dark) at room temperature until use. Once reconstituted in appropriate solvents, the MassPREP OST Standard solution is stable for one week when stored at 4 °C. Stability can be extended by freezing samples at -20 °C.

## V. ORDERING INFORMATION

| Description | Particle Size | Pore Size | Dimension | Part No. |
|---|---|---|---|---|
| ACQUITY UPLC OST C18 * | 1.7 µm | 135 Å | 2.1 x 50 mm | 186003949 |
| ACQUITY UPLC OST C18 * | 1.7 µm | 135 Å | 2.1 x 100 mm | 186003950 |
| Custom ACQUITY UPLC OST C18 * | -- | -- | -- | 186003951 |
| XBridge OST C18 | 2.5 µm | 135 Å | 2.1 x 50 mm | 186003952 |
| XBridge OST C18 | 2.5 µm | 135 Å | 4.6 x 50 mm | 186003953 |
| XBridge OST C18 | 2.5 µm | 135 Å | 10 x 50 mm | 186003954 |
| Custom XBridge OST C18 | -- | -- | -- | 186003955 |
| MassPREP OST Standard | | | 1 Vial | 186004135 |

** For use on Waters ACQUITY UPLC Systems*

*† MSDS information: Waters MassPREP OST Standard contains a non hazardous peptide in powder form. It is for laboratory use only; solely for purposes of scientific experimentation, analysis, research or development. It should be used only as directed and in accordance with good laboratory practice. It is non hazardous as defined by OSHA's Hazard Communication Standard, 29 CFR 1910.1200(c) and Appendix A and B, and are thus exempt from the requirements of the Hazard Communication Standard, including the requirement to supply MSDSs.*

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