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アプリケーションノート

Extraction of Oligonucleotides From Plasma Samples Across Multiple Species Using OligoWorks™ SPE Microplate Kit

Mary Trudeau, Matthew A. Lauber

Waters Corporation

Abstract

Oligonucleotide therapeutics are a key focus area for many drug developers today given their powerful ability to address disease biology at the level of gene transcription and translation with high target specificity and low toxicity. They are designed with specific types of modifications that enhance protein binding, half-life, and cellular uptake. Given their diversity, developing robust bioanalytical methods for oligonucleotide analysis can be quite challenging, and sample preparation is often its most challenging aspect due to the tight oligonucleotide-to-protein binding that occurs in biomatrices, often leading to low extraction recoveries and poor sensitivity. This work demonstrates use of the OligoWorks SPE Microplate Kit with RapiZyme™ Proteinase K Digestion to effectively extract oligonucleotides from plasma samples across multiple preclinical animal species including human. Using the starting protocol with no adjustments, oligonucleotide recoveries between 56–115% were obtained.

Experimental

Sample Preparation and Extraction

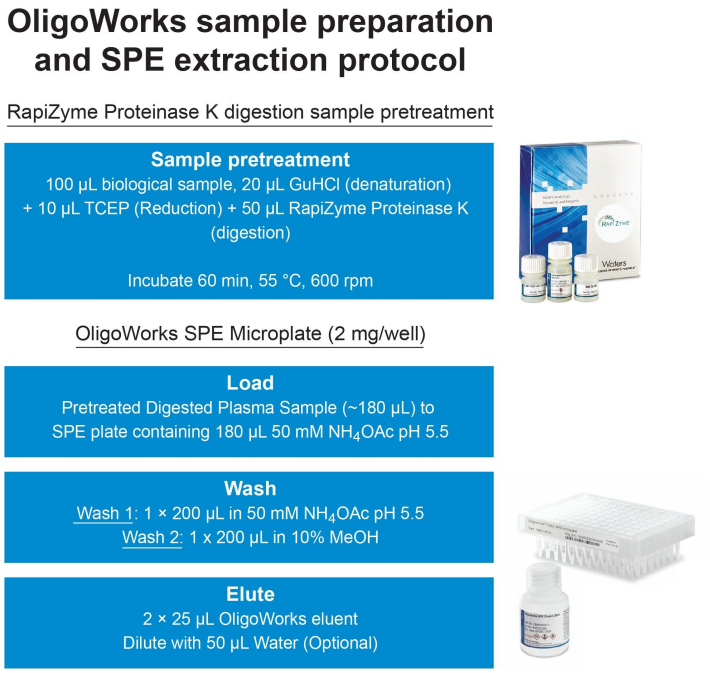

Figure 1. Graphical representation of the OligoWorks SPE Microplate Kit (p/n: 186010614) sample preparation protocol, with plasma sample digestion pretreatment using RapiZyme Proteinase K, followed by WAX SPE using the OligoWorks SPE microplate. LC-MS analysis of plasma sample extracts was performed with an ACQUITY™ UPLC™ I-Class PLUS (FL) coupled to a Waters Xevo™ TQ-XS Tandem Quadrupole Mass Spectrometer using multiple-reaction monitoring mode (MRM). Chromatographic separation was achieved ACQUITY UPLC Premier Oligonucleotide C_{18} Column, 130 Å, 1.7 µm, 2.1 x 50 mm (p/n: 186009484).

Results

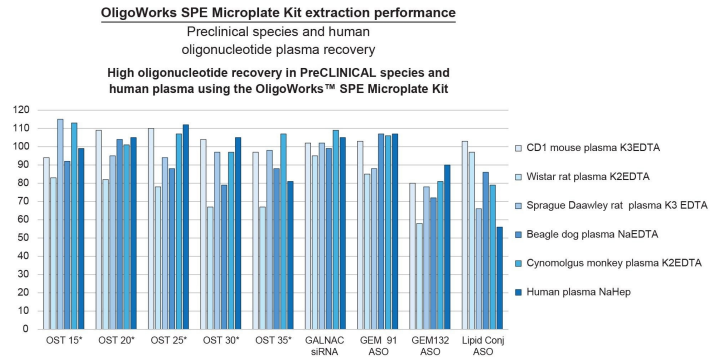

Figure 2. OligoWorks SPE Microplate Kit extraction performance (no internal standard correction) demonstrating high plasma recoveries (1-hour RapiZyme Proteinase K Digestion, 55 °C), for a diversity of oligonucleotides. *A 1:1 water dilution of digested plasma sample was applied prior to SPE loading. The 1:1 dilution minimized oligonucleotide loss (break-through) on SPE sample load, ensuring high SPE recovery.*

Ordering Information

Description	P/N
OligoWorks SPE Microplate Kit	186010614
ACQUITY Premier Oligonucleotide C_{18} Column, 130Å, 1.7 µm 2.1 × 50 mm	186009484
QuanRecovery™ with MaxPeak, 700 µL plate	186009184
Polypropylene cap mat round well for 96-well	186009452

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ACQUITY UPLC I-Class PLUS System <https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/acquity-uplc-i-class-plus-system.html>

Xevo TQ-XS Triple Quadrupole Mass Spectrometer <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/xevo-tq-xs.html>

RapiZyme Proteinase K Digestion Module <https://www.waters.com/nextgen/global/products/standards-and-reagents/rapizyme-proteinase-k-digestion-module.html>

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