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Note d'application

High Oligonucleotide Recovery From Liver Tissue

Kim Van Tran, Mary Trudeau, Matthew A. Lauber

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief provides guidance on the extraction of therapeutic oligonucleotides from tissue samples for quantitative analysis and demonstrates high oligonucleotide recovery from Liver Tissue.

Experimental

The following (Figure 1) summarizes the solvent-assisted tissue homogenization and oligonucleotide extraction workflow and protocol we use internally with our OligoWorks SPE Microplate Kit. Note we utilize a 1:10 ratio between the mg of tissue being homogenized and the total volumne (mL) of homogenization mix, including Rapizyme Proteinase K digestion reagents, buffer and solvent.

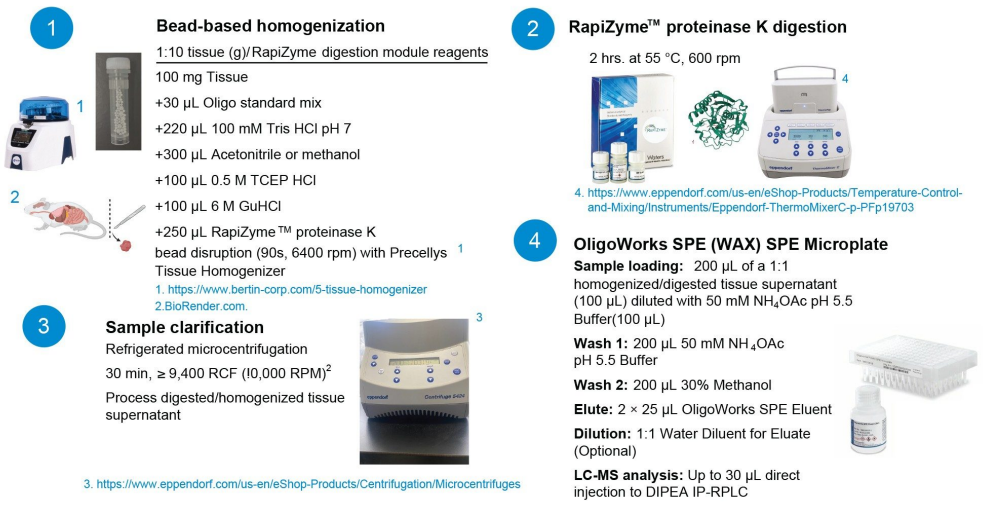

Figure 1. Oligonucleotide tissue extraction workflow and protocol using solvent assisted tissue homogenization and digestion with RapiZyme Proteinase K Digestion Module and OligoWorks SPE Microplate-2 mg/well.

Note: equipment referenced is what we use in our lab, but alternate equipment with equivalent capabilities may be used instead.

LC-MS Analysis

LC-MS ANALYSIS

UPLC	ACQUITY™ Premier BSM System FTN
MPA	1% HFIP (hwxafluoro-2-propanol) 0.1% DIPEA (N2N-Diisopropylethylamine) H_2O (Water)
MPB	0.75% HFIP 0.0375% DIPEA, 65% Acetonitrile (ACN)
Column sorbents	ACQUITY Premier Oligonucleotide C_{18} Column, 130Å, 1.7 µm 2.1 x 50 mm
Col temp.	50 °C
Sample temp.	10 °C
Inj. volume	5–15 µL
Purge solvent	90:10 H_2O:MeOH (Methanol)
Wash solvent	25:25:25:25 Water:MeOH:ACN:IPA
MS	**Xevo® TQ-Absolute**
Capillary (kV)	2.0
Desolvation temp.	500 °C
Desolvation flow	1000 L/Hr
Cone gas flow	150 L/Hr

Time (min)	Flow (mL/min)	%A	%B	Curve
0.00	0.6	95	5	6
3.25	0.6	77	23	6
3.75	0.6	10	90	6
4.1	0.6	10	90	6
4.25	0.6	95	5	6

Results and Discussion

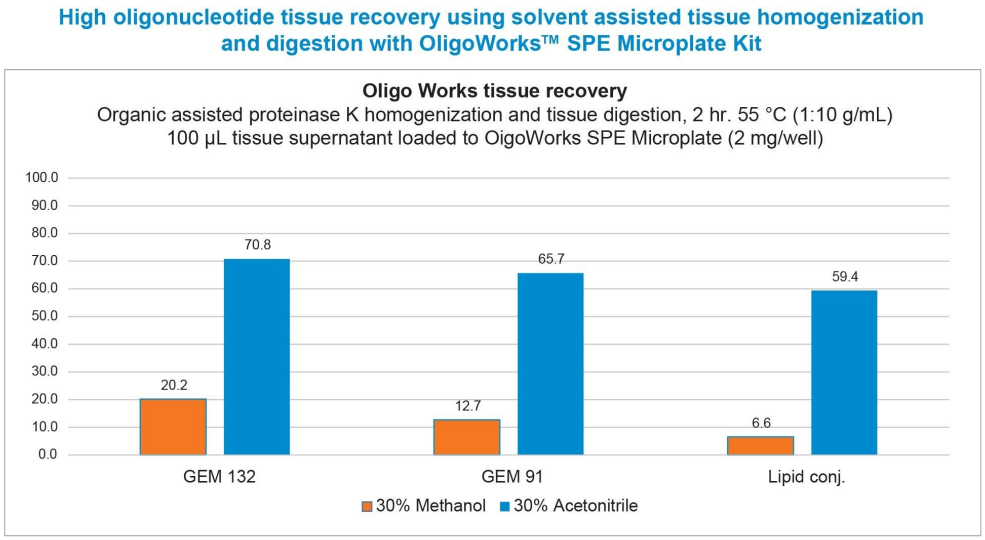

Figure 2. Demonstration of OligoWorks SPE Microplate performance, with >70% oligonucleotide recovery using 0.05 g tissue/0.5 mL Proteinase K Digestion Module reagents (homogenized and digested 2 hrs at 55 °C, 600 rpm), and 100 µL of tissue supernatant purified using the OligoWorks SPE Microplate Kit, containing OligoWorks RapiZyme Proteinase K Digest Module and OligoWorks SPE Microplate-2 mg/well.

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

Featured Products

ACQUITY Premier System <https://www.waters.com/waters/nav.htm?cid=135077739>

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

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