

High Oligonucleotide Recovery From Liver Tissue

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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 volume (mL) of homogenization mix, including Rapizyme Proteinase K digestion reagents, buffer and solvent.


1 **Bead-based homogenization**
 1:10 tissue (g)/RapiZyme digestion module reagents
 100 mg Tissue
 +30 µL Oligo standard mix
 +220 µL 100 mM Tris HCl pH 7
 +300 µL Acetonitrile or methanol
 +100 µL 0.5 M TCEP HCl
 +100 µL 6 M GuHCl
 +250 µL RapiZyme™ proteinase K
 bead disruption (90s, 6400 rpm) with Precellys 1
 Tissue Homogenizer
 1. <https://www.berlin-corp.com/5-tissue-homogenizer>
 2. BioRender.com.

2 **RapiZyme™ proteinase K digestion**
 2 hrs. at 55 °C, 600 rpm



4. <https://www.eppendorf.com/us-en/eShop-Products/Temperature-Control-and-Mixing/Instruments/Eppendorf-ThermoMixer-C-p-PFp19703>

3 **Sample clarification**
 Refrigerated microcentrifugation
 30 min, ≥ 9,400 RCF (10,000 RPM)²
 Process digested/homogenized tissue supernatant



3. <https://www.eppendorf.com/us-en/eShop-Products/Centrifugation/Microcentrifuges>

4 **OligoWorks SPE (WAX) SPE Microplate**
Sample loading: 200 µL of a 1:1 homogenized/digested tissue supernatant (100 µL) diluted with 50 mM NH₄OAc pH 5.5 Buffer (100 µL)
Wash 1: 200 µL 50 mM NH₄OAc pH 5.5 Buffer
Wash 2: 200 µL 30% Methanol
Elute: 2 × 25 µL OligoWorks SPE Eluent
Dilution: 1:1 Water Diluent for Eluate (Optional)
LC-MS analysis: Up to 30 µL direct injection to DIPEA IP-RPLC




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 (hexafluoro-2-propanol) 0.1% DIPEA (N,N-Diisopropylethylamine) H ₂ O (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 ₂ O: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

High oligonucleotide tissue recovery using solvent assisted tissue homogenization and digestion with OligoWorks™ SPE Microplate Kit

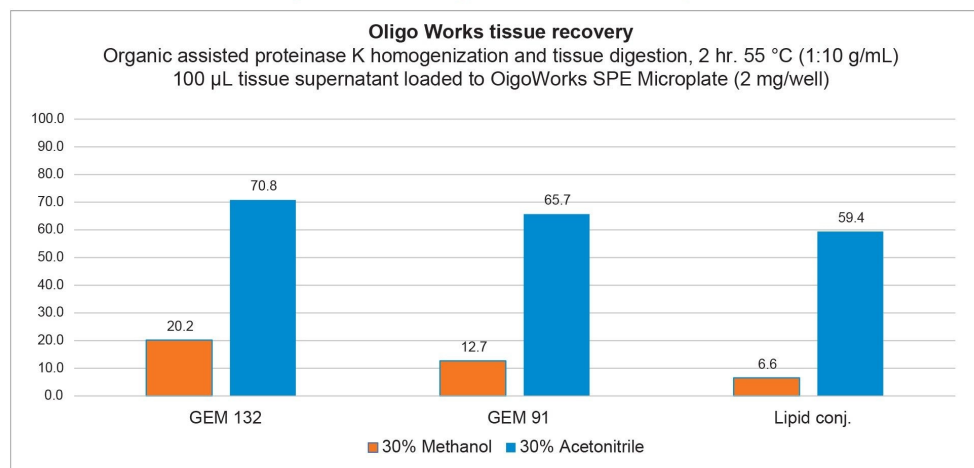


Figure 2. Demonstration of OligoWorks SPE Microplate performance, with >70% oligonucleotide recovery using 0.05 g tissue/0.5 mL Proteinase K Digestion Module reagents (homogenized and digested 2 hrs at 55 °C, 600 rpm), and 100 µL of tissue supernatant purified using the OligoWorks SPE Microplate Kit,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

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[ACQUITY Premier System <https://www.waters.com/waters/nav.htm?cid=135077739>](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>](https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/xevo-tq-absolute.html)

720008270, April 2018



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