Oligonucleotides

System preparation for optimized oligonucleotide analysis

Standard Operating Procedure (SOP)

Reagents

1. Either fresh Milli-Q® water 18.2 MΩ or Thermo Scientific™ ChromaCare™ UHPLC/MS
   Catalog number: W8-1
2. Methanol, UHPLC-MS, Thermo Scientific™
   Catalog number: A458-1
3. Triethylamine, 99%, pure, Thermo Scientific™
   Catalog number: 157911000
4. 1,1,1,3,3,3-Hexafluoro-2-propanol, 99.9%, for spectroscopy, Thermo Scientific™ (HFIP)
   Catalog number: AC293410500
5. Tributylamine, 99%, Thermo Scientific™ (TBA)
   Catalog number: AC139321000
6. High purity low metal ion acetic acid
7. Thermo Scientific™ 9 mm Plastic Screw Thread Vials
   Catalog number: C4000-11
8. Thermo Scientific™ Dionex™ Methanesulfonic Acid
   Catalog number: 080388
9. Thermo Scientific™ DNAPac™ RP Columns
   Catalog number: 088923 (2.1 × 100 mm)
10. Thermo Scientific™ SMART Digest™ RNase Kits
    Catalog number: 60120-101
11. Acetonitrile, UHPLC-MS, Thermo Scientific™
    Catalog number: A956-1 [not optima]

All products are from Fisher Scientific and Thermo Fisher Scientific.

UHPLC system cleaning

- 100 mM Methane sulphonic acid, ideally overnight or a minimum of 2 hours.

mRNA digest

- EDTA is not necessary, 20–50 µg mRNA is required.
- The volumes can all be reduced to use less mRNA sample.
- A strong magnet is needed to remove the beads.
- Heater shaker at 37 °C on 1,400 rpm.

Component	Final concentration in 200 µL
mRNA	0.5 mg/mL
EDTA	0–5 mM
RNAse T1	2.5 µL
Buffer	200 µL total

* Time depends on the mRNA sample. Unmodified [5 min], modified [15–30 min].

Chromatography

Eluents	
Pump flow	0.3 mL/min A = 20 mM TEA, 80 mM HFIP B = 20 mM TEA, 80 mM HFIP, 20% Acetonitrile
Gradient	T = 0–5% B T = 0.5–5% B T = 40–20% B T = 41–80% B T = 45–80% B T = 46–5% B T = 52 End
Column oven	50 °C
Sampler	No wash
If UV	260 nm

Chromatography (continued)

MS conditions	
Method duration	52 min
Spray voltage	Negative ion 2,500
Sheath gas	35
Aux gas	10
Ion transfer tube temp	320 °C
Vaporizer temp	300 °C
Application mode	Peptide
Pressure mode	Standard
Expected peak width	15 sec
MS scan resolution	120,000
Scan range	*m/z* 450–3,000
AGC target %	200
Microscans	2
RF lens	38
Polarity	Negative
Include charge states	2–20
Exclusion duration	8 sec
Scan ddMS	
HCD stepped energy	18/20/22
Resolution	30,000
Scan range	150–2,000
AGC target %	50
Microscans	2

Thermo Scientific BioPharma Finder software

- Data analysis was performed in Thermo Scientific™ BioPharma Finder™ v5.0 software.
- Data analysis used the basic default method in the oligonucleotide sequencing module. To identify large fragment ions, the maximum oligonucleotide mass was set to 25,000 Da, minimum confidence at 0.5 and mass accuracy at 10 ppm.
- The ribonuclease selection was set to RNase T1, specificity level set at 'strict' and phosphate location was set at 'none'. Phosphorylation and cyclic phosphorylation were set as variable modifications of the 3' terminal in the sequence manager containing the RNA sequence. (Most fragments found are cyclic phosphorylation.)

Learn more at **thermofisher.com/oligonucleotides**