Advion Interchim scientific®

ThermoFisher SCIENTIFIC

Sample preparation

SMART Digest OligoSelect Kit

Product manual

thermo scientific

Introduction

Thermo Scientific™ SMART Digest™ OligoSelect™ Kit (Part No. 60190-101)

The combination of thermally stable Thermo Scientific™ SMART Digest™ Soluble Proteinase K Kit and Thermo Scientific™ SMART Digest™ OligoSelect™ Magnetic WAX (Weak Anion Exchange) Beads enables simultaneous protein digestion and oligonucleotide isolation in a single well for rapid, reproducible recovery of oligonucleotides from a variety of biological matrices.

Table 1. SMART Digest OligoSelect kit components

Component	Format
SMART Digest soluble Proteinase K kit	0.5 mL, 20 mg/mL
Thermo Scientific™ SMART Digest™ Proteinase K Digest/Capture Buffer	25 mL optimized buffer (proprietary materials, tris based)
SMART Digest OligoSelect magnetic WAX beads	5 mg resin/well, weak anion exchange
Thermo Scientific™ SMART Digest™ Oligo Elution Buffer	25 mL WAX elution buffer (proprietary materials, water, MeOH, TEA)

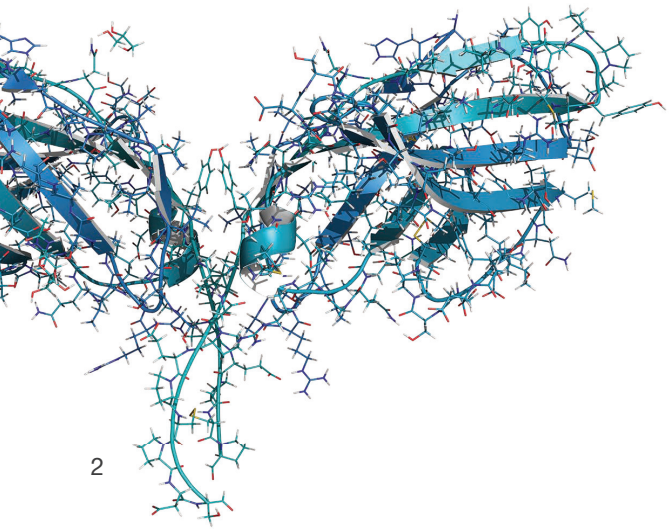
Best practices for reliable performance

- Use analytical reference standards to benchmark extraction efficiency. Spike samples pre- and post-pretreatment and after extraction to pinpoint where losses occur.
- Use low-adsorption plastic consumables—oligonucleotides can adsorb to many plastics and most metals, significantly affecting recovery. Avoid using glass vials.
- Use plastic or borosilicate glass for solvent bottles to minimize sodium adducts and beware of lot-to-lot variability of organic solvents.

Materials recommended, but not included

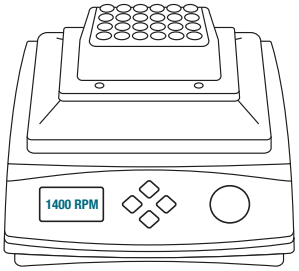
- A heater/shaker capable of providing uniform heating (e.g. Eppendorf ThermoMixer C with heated lid)
- Low adsorption deep well plates (and Thermo Scientific™ Matrix™ SepraSeal™ Capping System (Part No. 4463) or microcentrifuge tubes (typical reaction volumes range from 200-800 µL)
- Wash solution 1 (e.g. 50 mM ammonium acetate pH 5.5)
  - For 100 mL add the following to an appropriate container
    - 385 mg ammonium acetate
    - 100 mL H_2O
    - 57 µL glacial acetic acid
    - When not in use, store at 4 °C for up to 5 days
- Wash solution 2 (e.g. 25-50% methanol)*
  - For 100 mL add the following to an appropriate container
    - 30 mL methanol
    - 70 mL water
    - Store at room temperature for up to 6 months

*30% methanol is standard. If looking to improve recovery or reduce matrix effects, it is recommended that a variety of concentrations of organic solvent for the Wash solution 2 be evaluated as part of method development (e.g. compare 25, 50, and 75% MeOH). If desired, acetonitrile can be evaluated as an alternative to methanol.



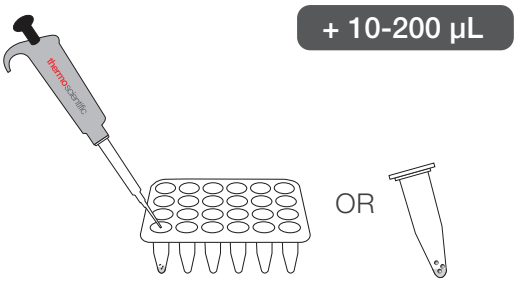
Protein digestion procedure

1 Setup/equilibrate heater



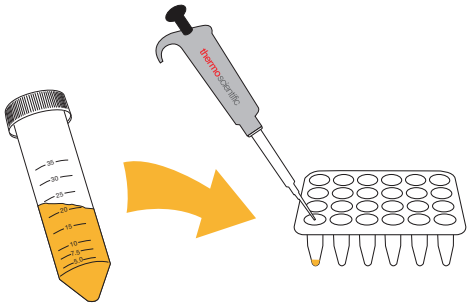
Create a method setting the heater/shaker temperature to the desired temperature (70 °C). Start this method and allow the temperature to reach equilibrium for at least 5 minutes before adding samples.

2 Add samples to well plates



Carefully add samples (10-200 µL) to low bind deep well plates or centrifuge tubes.

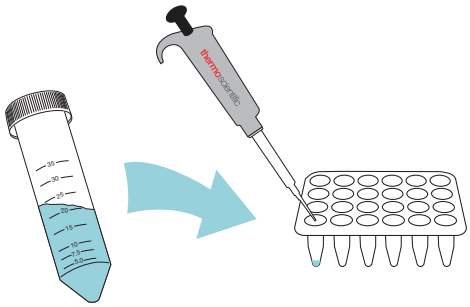
3 Dilute samples 4-fold



Dilute the samples 4-fold using SMART Digest Proteinase K digest/capture buffer (e.g. for 50 µL of sample, add 150 µL SMART Digest Proteinase K digest/capture buffer).

- When applicable, add internal standard.

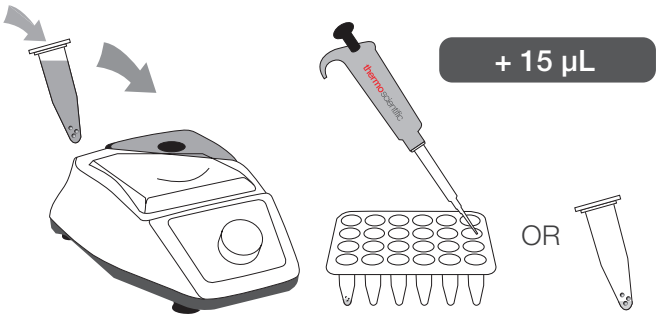
4 SMART Digest soluble Proteinase K kit



Add 5 µL of SMART Digest soluble Proteinase K to each sample well.

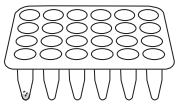
Protein digestion procedure (continued)

5 Re-suspend and aliquot



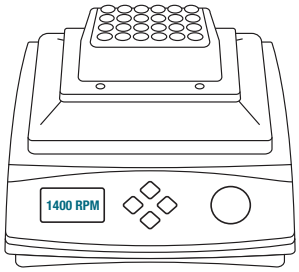
Vortex the bottle containing SMART Digest OligoSelect magnetic WAX beads thoroughly, then add 15 µL of beads to each well. For multi-well dispensations, the resin should be mixed before every draw step.

6 Cap/seal the samples



Cap or seal the samples, making sure all samples are tightly sealed.

7 Insert plate



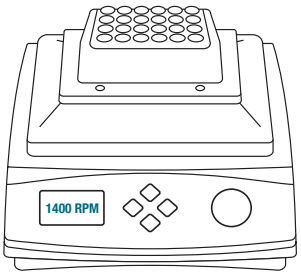
Place samples firmly into the heating block.

- If the equipment used is equipped with a lid, fasten the lid.

Protein digestion procedure (continued)

8

Digest samples for 15-90 minutes at 70 °C



15-90 min

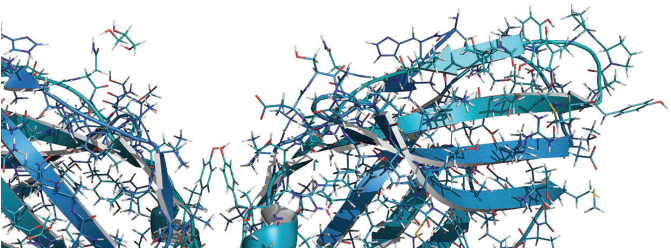
70 °C

Digest samples for 15-90 minutes at 70 °C. To keep the WAX beads in solution it is recommended to set the shaker to 1400 rpm.

- The optimal digestion time will vary widely depending on the format being used due to the time it takes for samples to reach the optimal digest temperature (~70 °C).
- When using microcentrifuge tubes or PCR plates, samples frequently digest in 15 minutes or less. When using fully loaded deepwell plates, samples frequently digest in 15 minutes but it may take time for samples to come to temperature. This can be validated using the procedure below.

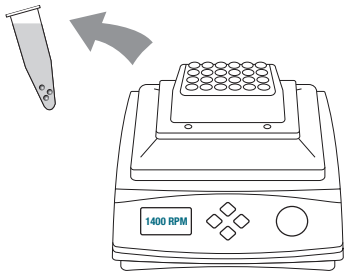
Digestion optimization

- To determine optimal digestion time, prepare eight identical samples, as described above, using a relatively high, known analyte concentration.
- Digest the samples according to the *Protein digestion procedure* and periodically remove one of the samples from the well or tube (e.g. every 5 to 15 minutes).
- Perform the appropriate *Post digestion process*.
- Analyze the samples to determine the extent of digestion.



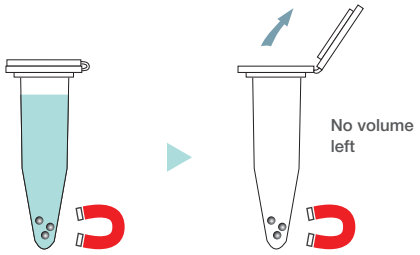
Post digestion procedure

1 Remove



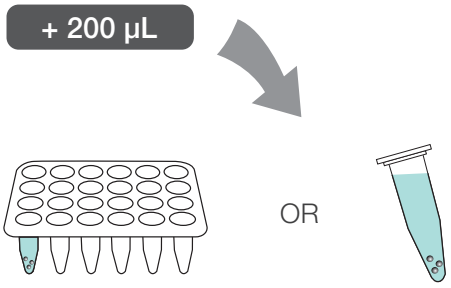
Following digestion, remove the samples from the heater/shaker and allow them to cool to room temperature (e.g. for 5 minutes).

2 Magnetize



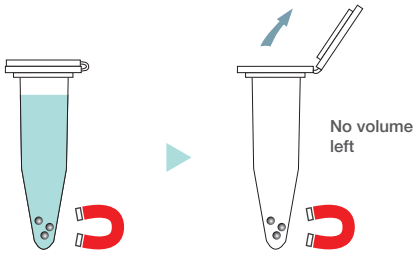
- Mix thoroughly.
- Place magnet to side of well/tube. The beads will be held in place.
- Following magnetization, uncap well/tube and aspirate all supernatant and discard.

3 Add wash solution 1



Add 200 µL wash solution 1 (e.g. ammonium acetate) to each well.

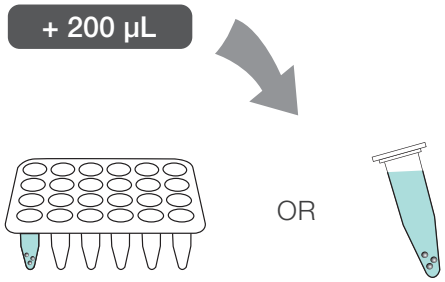
4 Magnetize



- Mix thoroughly.
- Place magnet to side of well/tube. The beads will be held in place.
- Following magnetization, uncap well/tube and aspirate all supernatant and discard.

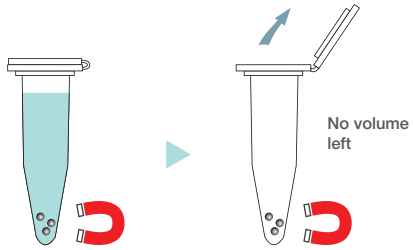
Post digestion procedure (continued)

5 Add wash solution 2



Add 200 µL wash solution 2 (e.g. 30% methanol) to each well.

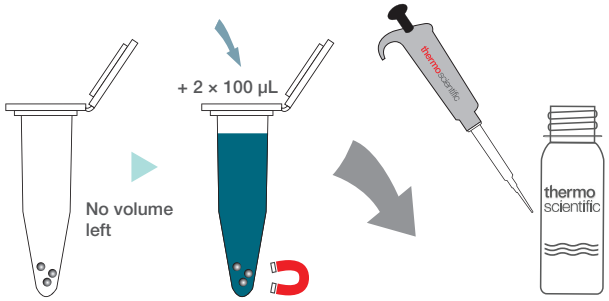
6 Magnetize



- Mix thoroughly.
- Place magnet to side of well/tube. The beads will be held in place.
- Following magnetization, uncap well/tube and aspirate all supernatant and discard.

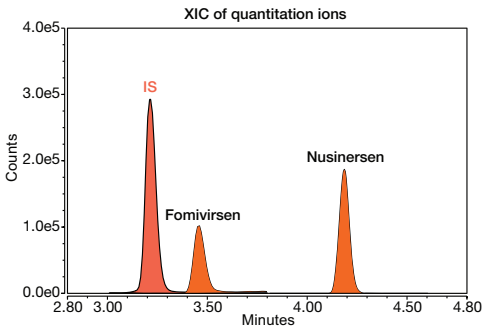
If necessary, repeat steps 3-6 to reduce background noise.

7 Elute



- Add 100 µL elution buffer to each well, mix thoroughly, place a magnet to the side of each well/tube, uncap well/tube, aspirate, and collect all the eluent.
- Repeat this step 1 more time.
- If the oligonucleotide being analyzed is unstable at high pH, samples can be neutralized using a 1:1 dilution with 1M ammonium acetate.
- The extraction procedure can be automated with a suitable magnetic bead automation system such as the Thermo Scientific™ KingFisher™ platform.

8 Analyze



- Collect eluent/sample and analyze.
- Due to the high organic concentration of the eluent, it may be beneficial to dilute samples 1:1 with water for improved sample stability in the autosampler as methanol may evaporate over time.
- If performing hydrophilic interaction liquid chromatography (HILIC) samples may require 1:1 dilution with acetonitrile prior to analysis.

Product information

Operational specifications

Table 2. Operational specifications

Feature	Range	Recommended
Sample volume	5-500 µL	5-200 µL
Digestion temperature	30–70 °C	70 °C
Wash solution 1	200 µL slightly acidic buffer	Ammonium acetate
Wash solution 2	200 µL of aqueous organic	30% MeOH
Elution	100-800 µL	2 × 100 µL

Recovery optimization

When analyzing oligonucleotides such as ASOs and siRNAs, variability in recovery can occur due to differences in structure, size, and chemical modifications. Method adjustments can fine-tune recovery and reduce matrix effects.

- Modifying the organic content of *Wash solution 2,* if looking to improve recovery or reduce matrix effects, it is recommended that a variety of concentrations of organic solvent for the Wash solution 2 be evaluated as part of method development (e.g. compare 25, 50, and 75% MeOH). If desired, acetonitrile can be evaluated as an alternative to methanol.
- Vary the volume of magnetic beads, try varying the volume of magnetic beads to as low as 7.5 uL per sample and up to 30uL per sample to determine optimal bind/recovery conditions.
- Increase total wash volume or steps if matrix effects remain high or if detergent was used.
- Increase elution volume or add an extra elution step to recover difficult analytes.
- Dilute eluate with water before LC-MS to better match ion-pairing conditions and reduce matrix suppression.
- Tissue or cell-based sample adjustments - for tissues, homogenization can be performed in the SMART OligoSelect digestion buffer followed by addition of SMART Digest soluble Proteinase K and digestion at 70 °C for 15 minutes (if working with tubes) or 90 minutes (if working with deep well plates). If using ceramic beads for the homogenization, these should be removed prior to addition of the SMART Digest OligoSelect magnetic WAX beads to prevent pulverization of the beads. As an option, 30% acetonitrile can be added to enhance homogenization. If 30% acetonitrile is added to the homogenization step, the sample should be diluted 2-fold after digestion to maintain recovery during the extraction step.

Product storage

All the SMART Digest OligoSelect kit materials are stable at room temperature for at least 1 month. For long term storage, store SMART Digest soluble Proteinase K kit, SMART Digest Proteinase K digest/capture buffer, SMART Digest oligo elution buffer, and SMART Digest OligoSelect magnetic WAX beads at 4 °C.

Ordering information

Description	Quantity	Cat. no
SMART Digest OligoSelect kit	Each	60190-101

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