Sample preparation

Thermo Scientific SMART Digest RNase Kits

Use and care instructions

Introduction

SMART Digest Ribonucleases (RNases) are digested using the same proven technology of Thermo Scientific™ SMART Digest™ RNase kits to provide simple, fast and highly reproducible digestions of ribonucleic acid (RNA) in automation compatible formats.

Characterization of sequence identity and purity is a critical aspect of RNA drug development. While sequencing methods have been the mainstay of sequence analysis for decades, LC-MS is uniquely suited to enable sequence identification as well as characterization of modifications to the base, sugar and backbone linkages. Furthermore, LC-MS allows for direct analysis, improved sensitivity over Sanger sequencing, and faster turnaround times than NGS. However, LC-MS analysis of RNA therapeutics typically requires the cleavage of RNA into fragments using ribonucleases.

Ribonucleases are highly efficient enzymes capable of digesting RNA in seconds, requiring methods to quickly quench the reactions in order to avoid over digestion. Typical approaches utilize the addition of enzyme denaturing reagents or inhibitors, such as aurintricarboxylic acid (ATA). However, these reagents are unreliable, can induce modifications to the digestion products and interfere with chromatographic analysis. This has been seen with ATA which polymerizes quickly in aqueous solutions.

To overcome these issues, the Thermo Scientific™ SMART Digest™ Immobilized Ribonuclease Magnetic Beads consist of enzymes covalently linked to magnetic beads allowing for simple and efficient termination of the reaction via bead removal. Additionally, the use of magnetic beads allows for the automation of digestion protocols.

Materials recommended but not provided

- Heater/shaker capable of providing uniform heating
- PCR strips, microcentrifuge tubes and deepwell plates can be used with this workflow
- Typical reaction volumes range from 200-1500 µL

Ordering information

Description	Format	Quantity	Cat. no
SMART Digest RNase T1 Mag Bulk kit (using magnetic beads)	Magnetic resin	Each	60120-101
SMART Digest RNase A MAg Bulk kit	Magnetic resin	Each	60120-102

RNA digestion procedure

1. Sample preparation – Dilute the RNA sample in the chosen digestion buffer (e.g. 50 mM Tris Buffer) to a final volume of no more than 200 µL per sample.
   - 50 mM Tris buffer is provided. As needed alternative buffers or additional additives can be used.
2. Create a method setting the heater temperature to the desired temperature (30 – 70 °C). Start this method and allow the temperature to reach equilibrium for at least 5 minutes before adding samples.
   - It is not recommended to exceed 50 °C for Ribonuclease T1 products as studies have shown operation at temperatures exceeding 50°C significantly decreases digestion efficiency.
3. Carefully add samples to deepwell plates, PCR tubes, or centrifuge tubes.
4. Add SMART Digest Ribonuclease Resin to the samples.
   - It is recommended to add 2.5 – 15 µL of enzyme bead slurry to each reaction; however, multiple factors should be considered when determining enzyme amount, such as sample concentration and complexity, extent of digestion desired, etc.
5. Cap or seal the samples, making sure all samples are tightly sealed.
6. Place samples firmly into the heating block.
   - If the equipment used is equipped with a lid, fasten the lid.
7. Following digestion, remove the samples from the shaker and immediately quench the reaction:
   - For RNA mapping (or partial digestion) studies, it is critical to quickly remove the resin to prevent over-digestion.
     - This may be done using a magnet to either completely removes the resin or pulling the resin to the bottom of the sample allowing the supernatant to be quickly extracted.
8. Once the supernatant has been removed, transfer the appropriate amount to a deepwell plate and dilute as necessary prior to analysis.
   - Any diluent can be used as long as it is compatible with the corresponding analysis procedures.
9. Analyze oligonucleotide products by the desired method.

Operational specifications

Description	
Sample volume	Typically 200 µL, but up to 1.5 mL*
Digestion buffers	50mM Tris buffer (pH 7) provided
RNA sample concentration	≤2.5 mg/mL (max 500 ug/reaction)
Operating temperature (°C)	30–70
Storage temperature (°C)	-20 (long term), 4 (short term, ≤14 days)

* If validated, resin can be added directly to the sample

Optimization

All RNA samples vary with regards to optimal digestion parameters; adjust concentrations, temperature and incubation time accordingly.

Typically, in order to optimize digestion time, a known, relatively high, concentration of native analyte in the matrix of operation is used for a time-course experiment: The sample is placed into each of 8 wells containing the immobilized enzyme. The samples are then as a group added to the heat/shaker. Periodically, a sample is removed, the beads extracted and the samples analyzed.

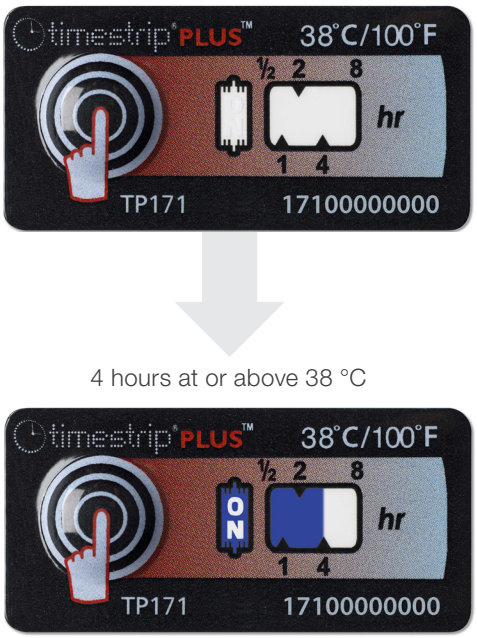
For SMART Digest Ribonuclease T1 (SDRT1), it is not recommended to exceed an operating temperature greater than 50 °C. Studies have shown a significant decrease in digestion activity above this temperature with an optimal range of 30–50 °C. SDRT has been determined to maintain at least 70% of its original digestion activity when incubated for 30 minutes at these temperatures prior to RNA addition to the samples.

For SMART Digest Ribonuclease A (SDRA), studies suggest that digestions may be run up to 90 °C with moderate decreases in digestion activity (70% after 30 minutes at 90 °C) and up to 70 °C with no decrease in activity.

Product storage

Store the enzyme materials at -20 °C. Store SMART Digest buffer at either 4 °C or -20 °C. If desired all other materials can be stored at room temperature.

Each kit will come with a WarmMark®2 Temperature Indicator. This indicator tracks how long the kit has been at or above 38 °C up to 8 hours by irreversibly turning from white to blue. Should the time indicator reach 8 hours, please contact **techsupport.ccs@thermofisher.com** in order to determine the shipping conditions experienced and ascertain the functionality of the kit. Below is an example of how the temperature labeling works.



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