**RapiGest SF Surfactant**

US Patent No: 7,229,539 and US 8,580,533

## CONTENTS

**I. INTRODUCTION**

*RapiGest™ SF* is a reagent used to enhance in-gel\(^1\) and in-solution\(^2-6\) enzymatic digestions of proteins. Recent investigations also support its use to solubilize glycoproteins prior to deglycosylation with enzymes such as PNGase F\(^7\). *RapiGest SF* helps solubilize proteins making them more susceptible to enzymatic cleavage without significantly inhibiting enzyme activity and is heat stable for higher temperature digestions. Unlike other commonly used denaturants (e.g., SDS or Urea), *RapiGest SF* does not modify peptides or suppress endoprotease activity. *RapiGest SF* is compatible with enzymes such as Trypsin, PNGase F, Lys-C, Arg-C, Asp-N, Glu-C and other enzymes. This reagent is easily removed after use allowing MALDI-TOF MS, LC or LC/MS analyses of digested samples.

**II. STORAGE AND STABILITY**

The lyophilized powder is stable at room temperature until the expiration date printed on the label (i.e., 3 years after packaging). Once reconstituted in high purity water or a buffer (pH 7–10) the solution is stable for one week when stored at 2–8 °C. Long term storage of frozen aliquots is possible but not recommended due to potential solubilization issues of *RapiGest SF* or storage buffer.

*Note: RapiGest SF hydrolyzes in acidic solutions (half life 8 min. at pH 2 and 60 min. at pH 3)*
III. RECONSTITUTION OF RapiGest SF POWDER

Shown in Table 1, are the volumes required to reconstitute the 1 mg RapiGest SF powder in water or buffer to obtain preferred concentrations. If Mass Spec analysis is required, the recommended buffer is 50 mM Ammonium Bicarbonate (NH₄HCO₃). Alternative buffers, such as 10 mM Tris-HCl or 25 mM sodium phosphate, are also RapiGest SF compatible.

Table 1: Reconstitution of RapiGest SF Powder

<table>
<thead>
<tr>
<th>Volume of buffer added to RapiGest SF vial</th>
<th>RapiGest SF concentration (w/v)</th>
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<tbody>
<tr>
<td>1 mL</td>
<td>0.1%</td>
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<tr>
<td>500 μL</td>
<td>0.2%</td>
</tr>
<tr>
<td>200 μL</td>
<td>0.5%</td>
</tr>
<tr>
<td>100 μL</td>
<td>1%</td>
</tr>
<tr>
<td>50 μL</td>
<td>2%</td>
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</table>

The recommended concentration is 0.1% (w/v) RapiGest SF. Hydrophobic proteins may require higher RapiGest SF concentrations (see note for recommendations for working with proteolytic resistant or hydrophobic proteins).

IV. SUGGESTED PROCEDURE FOR IN-SOLUTION DIGESTIONS

(Note: Modifications to this suggested method, for example increased digestion times, may be necessary depending upon the target proteins.)

1. Suspense the 1 mg of lyophilized RapiGest SF powder in 1 mL of 50 mM Ammonium Bicarbonate (NH₄HCO₃) to give 0.1% (w/v).
2. Suspense protein pellet in the 0.1% RapiGest SF solution and vortex.
3. Add DTT to the protein sample to a final concentration of 5 mM.
4. Heat the sample at 60 °C for 30 minutes.
5. Cool the sample to room temperature.
6. Add Iodoacetamide to the sample to a final concentration of 15 mM and place the sample in the dark for 30 minutes.
7. Add enzyme for digestion (1:100 to 1:20, w/w).
8. Incubate the samples at 37 °C for (1 hr to overnight depending upon protein hydrophobicity) for optimum enzymatic digestion.

V. SUGGESTED PROCEDURE FOR IN-GEL DIGESTIONS

(Note: Modifications to this suggested method may be necessary depending upon the target proteins and applications.)

1. Excise protein spots from gel, wash with 30 μL of H₂O and leave for 15 min.
2. Slice gel spots into <1 mm³ cubes, transfer the gel pieces into a 1.5 ml microcentrifuge tube, add 30 μL of H₂O and leave at room temperature for 15 min.
3. Remove the supernatant, add 20 μL of 50% acetonitrile, mix, and leave for 15 min.
4. Remove the supernatant, add 30 μL of 100% acetonitrile, mix, and leave for 15 min.
5. Remove the supernatant, add 20 μL of 0.1 M NH₄HCO₃, mix, and leave for 5 min.
6. Add 30 μL of 100% acetonitrile, mix, and leave for 15 min.
7. Remove the supernatant and completely dry the gel pieces with a Speed Vac.
8. Add 50 μL of 10 mM DTT in 0.1 M NH₄HCO₃ and incubate at 56 °C for 45 min.
9. Remove the supernatant, add 30 μL of 55 mM iodoacetamide in 0.1 M NH₄HCO₃ and leave in the dark for 30 min. (Repeat steps 3–7)
10. Add 20 μL of 0.1% RapiGest SF solution in 50 mM NH₄HCO₃ and incubate at 37 °C for 10 min.
11. Remove excess solution and completely dry the gel pieces with a Speed Vac.
12. Gradually (single drop at a time) add trypsin (12.5 ng per μL in 50 mM NH₄HCO₃) to the gel slice until the gel is reswollen. Continue to incubate on ice for 45 min.
13. Remove excess solution, add 20 μL of 50 mM NH₄HCO₃ and incubate at 37 °C overnight.
VI. SUGGESTIONS FOR WORKING WITH PROTEOLYTIC RESISTANT OR HYDROPHOBIC PROTEINS

When dealing with hydrophobic proteins such as membrane proteins, the following modifications are recommended:
(See Ref. 3 for more information)

- Boil the protein/RapiGest SF mixture at ~ 100 °C for 5 minutes, cool the sample down before adding proteolytic enzymes.
- Proteins that are highly resistant to protease may require longer digestion times or higher RapiGest SF concentrations.

VII. SUGGESTED SAMPLE PREPARATION FOR LC AND LC/MS

1. Add TFA to the digested protein samples. The final TFA concentration should be approx. 0.5% (pH <2).
   (Note: Obtain high purity Trifluoroacetic acid (TFA). The purchase and use of sealed vials containing 1 mL of high purity TFA is recommended.)
2. Incubate the sample at 37 °C for 30 – 45 minutes. Slight cloudiness should be observed.
3. Centrifuge acid treated samples at 13,000 rpm for 10 minutes. The hydrolytic RapiGest SF by-products are water immiscible therefore, some precipitation may be observed.
4. Carefully transfer the solution to another microcentrifuge tube or autosampler vial.
5. Proceed with MALDI ToF, LC or LCMS analysis.

TIP AND TRICK

Sometimes an oily layer may be noticed. The oily layer contains the acid degradation product, since it is not water soluble and is less dense than water. This will only be observed when this byproduct gets precipitated as a transparent pellet at the top of an eppendorf tube after centrifugation. However, it is only observable when the sample is less concentrate and clean. When the protein sample is concentrated or in a complex matrix, this by-product is difficult to see, instead, it could co-precipitate along with other highly hydrophobic peptides or proteins. If this is a problem less RapiGest 0.1% (w/v) can be used as well as avoiding acidification.

VIII. SUGGESTED SAMPLE PREPARATION FOR GLYCOPROTEIN SAMPLES FOR RAPID DEGLYCOSYLATION

Note: This protocol is adapted from the GlycoWorks RapiGluor-MS N-Glycan protocol, 715004903.

1. The following protocol is based off a recommended glycoprotein concentration of 2mg/ml or 15 ug total protein.
2. Prepare a buffered solution of 5% (w/v) RapiGest by dissolving the contents of 1 vial (3 mg) of RapiGest in 60 μL of 5x GlycoWorks Rapid Buffer. Vortex to mix.
3. Add 15.3 μL of 18.2 M water into a tube (you can adjust this if you have smaller concentrations to start).
4. Dispense 7.5 μL of the 2 mg/mL glycoprotein solution into a tube.
5. Add 6 μL of buffered solution containing 5% (w/v) RapiGest SF. Aspirate and dispense to mix.
6. Heat denature this mixture for 3 minutes using a heat block such that the solution temperature reaches at least 90 °C.
7. Remove the tube from the heat block, allowing the deglycosylation mixture to cool at room temperature for 3 minutes.
8. Add 1.2 μL of recombinant, highly pure and active PNGase F, bringing the IgG concentration to 0.5 mg/mL. Aspirate and dispense to mix.
9. Incubate this mixture such that the solution temperature is maintained at 50 °C for 5 minutes.
10. Remove the tube from the heat block, allowing the deglycosylation mixture to cool at room temperature for 3 minutes.

TIP AND TRICK

- Accurate heating is very critical to complete deglycosylation
- The above protocol was developed to work in conjunction with HEPES buffer (250 mM HEPES and has been titrated with NaOH such that upon dilution to a 1x solution a pH of 7.9 is obtained).
V. ORDERING INFORMATION

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REFERENCES


Go to www.waters.com/rapigest for relevant Application Notes.