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# Product Information

**Low Artifact Digestion Buffer**

Catalog Number **EMS0011**
Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

Endogenous deamidation and oxidation of protein amino acids can be implicated in and be indicative of many diseases, protein turnover, development, and aging. The introduction of these modifications can often affect biological activity, half-life, and immunogenicity.

Current protein digestion workflows often introduce a significant amount of artifactual deamidation and oxidation which prohibits the measurement of the endogenous levels.

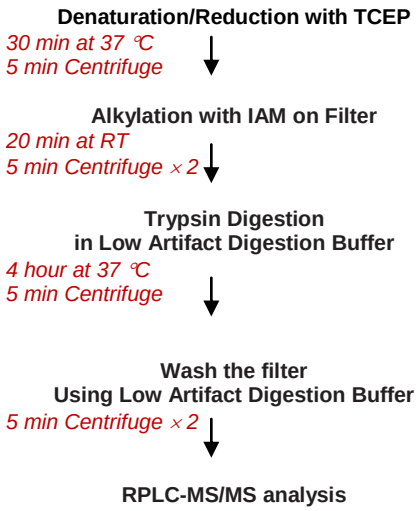
The Low Artifact Digestion Buffer allows protein digestion in less than 6 hours with minimized artifactual modifications such as deamidation and oxidation. The amount of oxidation and deamidation introduced during further downstream processing of biotherapeutic antibodies and proteins can be accurately determined.

The most commonly deamidated amino acid is Asn especially when followed by small amino acids such as Gly. The most commonly oxidized amino acids are Met, Cys, and to some extent Trp and His. Examples of deamidation and oxidation are shown in Figures 1 and 2, respectively.

### Reagents required but not provided

- Formic acid (FA), Catalog Number 399388
- Tris(2-carboxyethyl)phosphine (TCEP), solution 0.5 M, pH 7, Catalog Number 646547
- Iodoacetamide (IAM), 56 mg per vial, Catalog Number A3221
- SOLu-Trypsin, trypsin solution, 1 mg/mL, Catalog Number EMS0004
- 30 kDa filter, Microcon-30kDa Centrifugal Filter Unit with Ultracel-30 membrane, Catalog Number MRCF0R030,
- Urea, Catalog Number 51456

### Workflow




### Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Low Artifact Digestion Buffer is ready to use. Store unused portion at 2–8 °C for up to 2 years.

### Storage/Stability

The product is stable for at least 2 years at 2–8 °C.

**Procedures**

Preparation of Reagents

8 M urea solution – Dissolve 2.25 g of urea in 3 mL of water. The final volume will be about 4.5 mL.

100 mM TCEP solution, pH 7 – Dilute 200 μL of TCEP with 800 μL of 8 M urea

100 mM IAM solution – Dissolve one vial in 606 μL of 8 M urea for a 500 mM stock. Dilute 200 μL of stock solution with 800 μL of 8 M urea.

Trypsin Digestion

1. It is recommended to start with 50 μg or more of protein for the digestion. Volume of the sample solution should not exceed 100 μL. Minimum volume is 20 μL. Add water to bring the volume to 20 μL if necessary.
   Note: Exchange sample buffer to 8 M urea if formulation of the sample is known to interfere with reduction and/or alkylation.

2. Add 100 μL of 100 mM TCEP at pH 7. Incubate for 30 minutes at 37 °C while mixing at 300 rpm.
   Note: Mixing is optional.

3. Transfer the reduced sample to a Microcon-30kDa Centrifugal Filter Unit with Ultracel-30 membrane in a collection tube.
   Note: Do not use Amicon filter or any type of membrane other than Microcon Ultracel-30 membrane.

4. Centrifuge at 14,000 × *g* for 5 minutes*.

5. Discard the flow-through from the collection tube.

6. Add 100 μL of 100 mM IAM, vortex for 1 minute. Incubate while mixing for 20 minutes at ambient temperature in the dark.
   Note: Mixing is optional.

7. Centrifuge at 14,000 × *g* for 5 minutes.

8. Discard flow-through from the collection tube.

9. Add 150 μL of Low Artifact Digestion Buffer to each filter unit and centrifuge at 14,000 × *g* for 5 minutes*.

10. Repeat step 9 one more time.

11. Transfer the filter unit to new collection tube. Add 100 μL of Low Artifact Digestion Buffer to each filter unit.

12. Add enough 1 mg/mL SOLu-Trypsin solution to have an enzyme to protein ratio of ~1:10 and vortex for 1 minute.

13. Wrap the filter unit with Parafilm® M to prevent evaporation.

14. Incubate the filter unit at 37 °C for 4 hours while mixing at 300 rpm.
    Note: Mixing is optional.

15. Centrifuge the filter unit at 14,000 × *g* for 5 minutes*.

16. Add 40 μL of Low Artifact Digestion Buffer and centrifuge the filter unit at 14,000 × *g* for 3 minutes*.

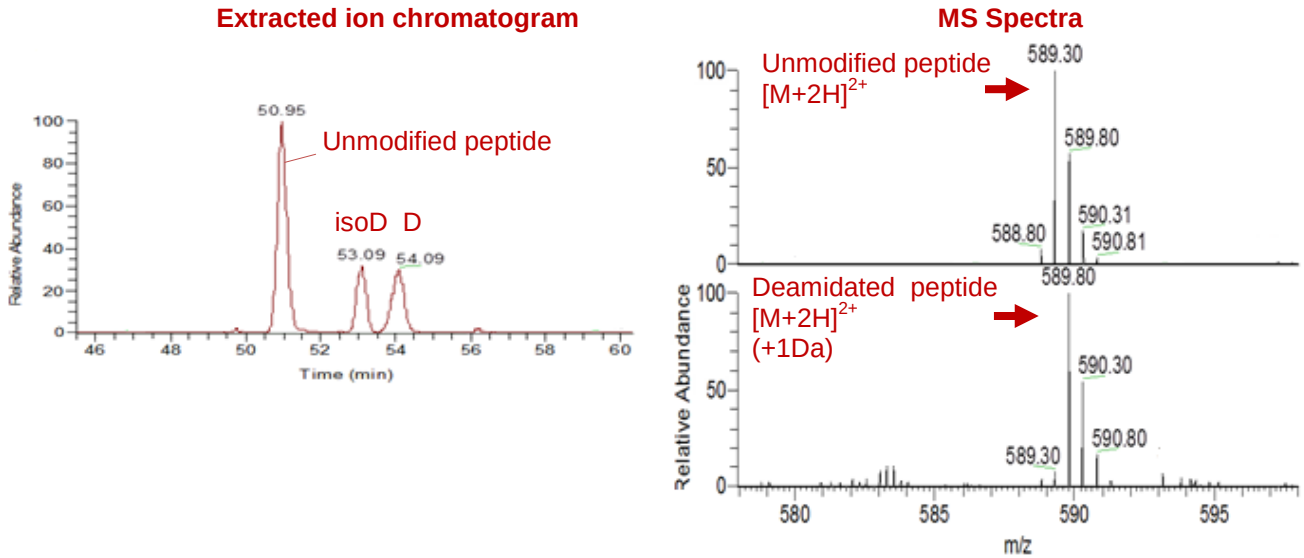
17. Repeat step 16 one more time.

18. Add 2 μL of neat formic acid to the filtrate. Vortex and spin briefly.

19. Run LC-MS/MS using a condition suitable to resolve modified peptides.
    Note: For deamidation quantification, peaks of Asn, Asp, and isoAsp products must be resolved chromatographically (1 Da mass difference). The oxidized and non-oxidized species will be distinguished easily (16 Da mass difference).

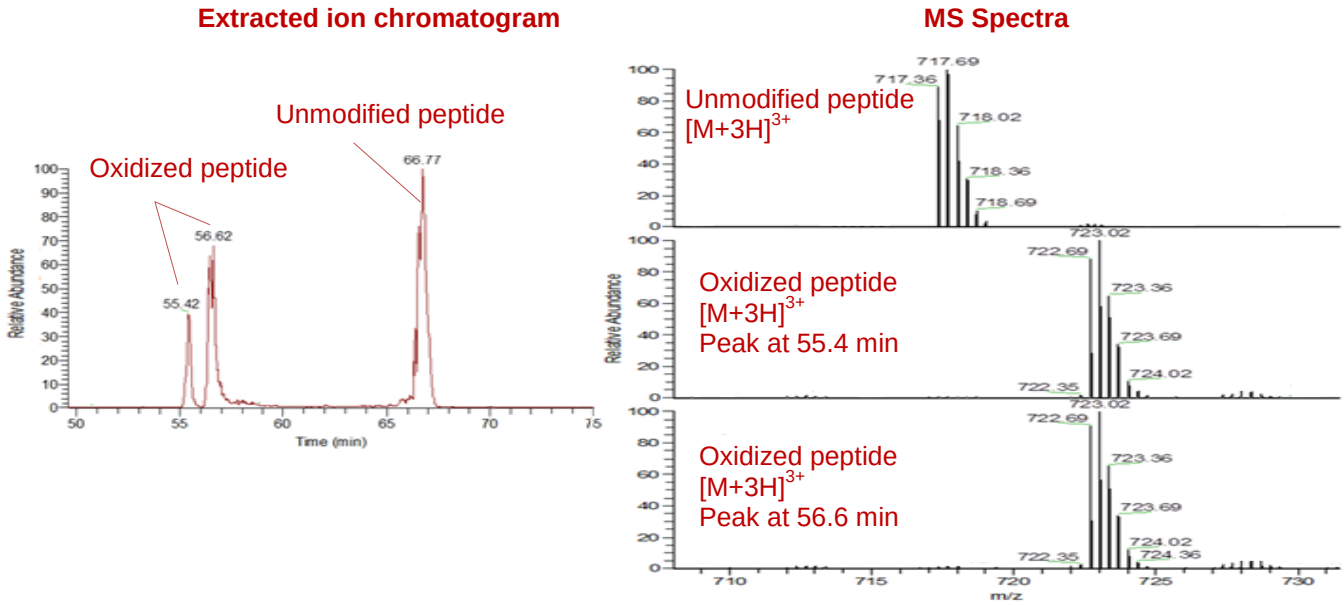
* – If solution has not fully passed through the filter, centrifuge for 2 more minutes. Do not centrifuge for additional time unless it is necessary; longer centrifugation time tends to increase oxidation.

**Figure 1**.
Example of deamidation




Extracted ion chromatogram and corresponding mass spectra of deamidation of Asn products of GYGVIFA**N**GNR($^{13}C_6{}^{15}N_4$) labeled peptide.

**Figure 2.**
Example of oxidation




Extracted ion chromatogram and corresponding mass spectra of oxidation of Met products of QATVGDINTERPG**M**LDFTGK peptide. Two peaks eluted at 55.4 and 56.6 minutes have the same mass and are diastereomers of the oxidized peptide.