



SPA-5001.01 – Sushi Δ 3 Anti-Sera

Introduction

The S Δ 3 peptide is a 34 amino acid peptide with a molecular weight of 3961.6 Da with the sequence:

His-Ala-Glu-His-Lys-Val-Lys-Ile-Lys-Val-Lys-Gln-Lys-Tyr-Gly-Gln-Phe-Pro-Gln-Gly-Thr-Glu-Val-Thr-Tyr-Thr-Cys-Ser-Gly-Asn-Tyr-Phe-Leu-Met

The sulfur group in the Cys residue can form disulfide bonds and create dimers in aqueous conditions. Here we use the formation of peptide dimers as an example protocol for using SPA-5001.01 – Sushi Δ 3 Anti-Sera in PAGE and Western Blotting experiments.

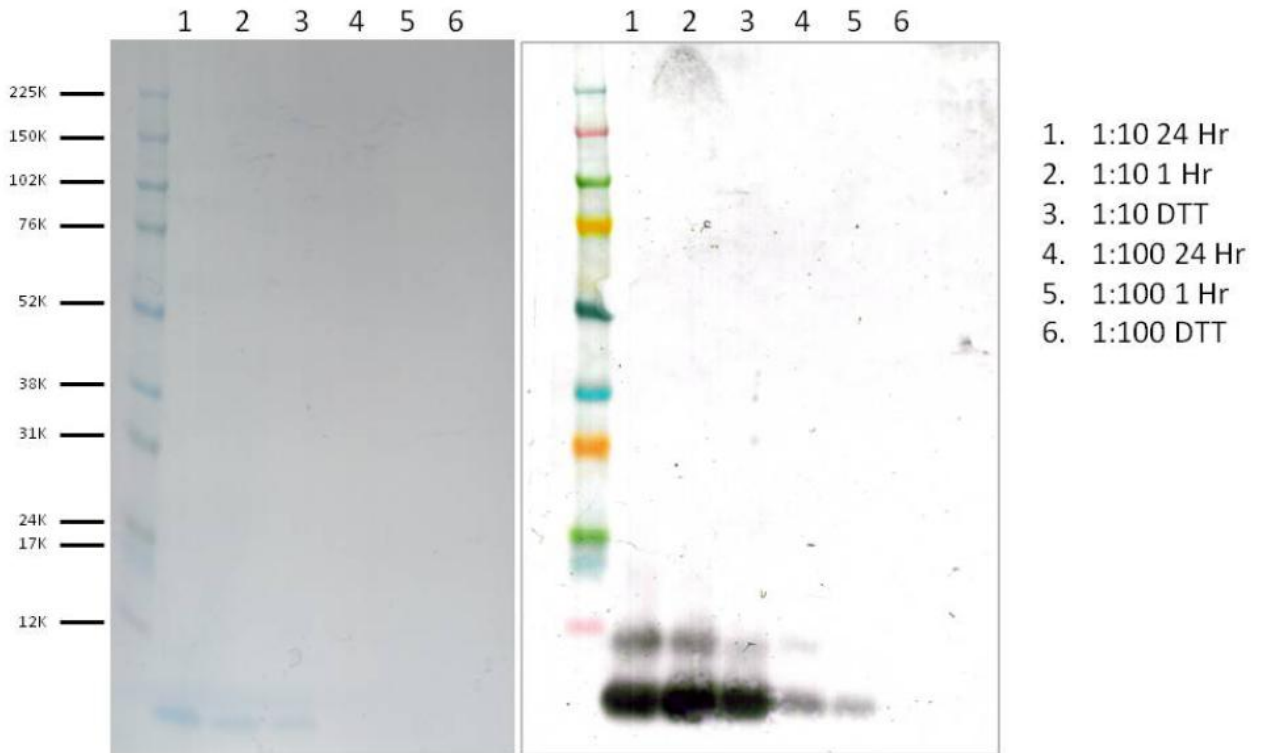
Protocol

1. Samples of 1 mg/ml S Δ 3 were diluted 1:10 and 1:100 in water and 5 mM dithiothreitol (DTT) either 1 hour or 24 hour prior to electrophoresis.
2. Samples were electrophoresed in duplicate using the NuPAGE SDS-PAGE gel system (Life Technologies) according to manufacturer's specifications.
3. One half of the gel was Coomassie stained using ClearPAGE Instant Blue Stain (CBS Scientific) according to manufacturer's specifications.
4. The other half of the gel was transferred to nitrocellulose for Western Blotting.
 - a. The transfer was done with the NuPAGE transfer system using an XCell II Blot Module (Life Technologies) according to manufacturer's specifications.
 - b. The WesternBreeze Chromogenic Western Blot Immunodetection Kit (Invitrogen) was used for detection.
 - c. SPA-5001.01 - Sushi Δ 3 Anti-Sera was diluted 1:100 in the primary antibody diluent for the results shown.

PAGE Results

The left is the Coomassie-stained gel. The right is the Western Blot with SPA-5001.01

Dilution is indicated in the legend. Times indicate incubation time in water. DTT samples were incubated for 1 hour.

Notes

- The SΔ3 peptide runs at 4K in the monomer form and 8K in the dimer form.
- SΔ3 dimers are not visible and SΔ3 monomers are not quantifiable with Coomassie staining.
- The majority of SΔ3 remains in monomer form even after 24 hours of aqueous incubation.
- There is significant reduction in the dimer population after 1 hour of treatment with DTT.
- A 1:100 dilution of SPA-5001.01 is sufficient to detect <0.01 mg SΔ3.