Nanoxis
Membrane protein solutions

Application Note

Improve your sequence coverage and confident protein identifications

Multistep digestion on immobilized membrane preparations using LPI™ FlowCell

Summary

This application note clearly demonstrates the benefits of using a two-step digestion protocol on a membrane preparation immobilized in LPI™ FlowCell. New peptides are identified in the second digestion step leading to improved sequence coverage, an increased number of confident protein identifications and a completely new set of proteins not detected in the first digestion step. This greatly enhances the protein sequence information obtainable from a membrane preparation analysis.

Method

A WSS-1cell membrane preparation was immobilized in LPI™ FlowCell and subjected to a two-step digestion protocol. The first step consisted of a brief trypsin digestion. The second step consisted of a trypsin digestion either with or without a digestion enhancer in the form of an acid-labile detergent, PPS Silent™ Surfactant (Protein Discovery).

Results

We conclusively show that a second trypsin treatment of a previously digested sample gives higher protein sequence coverage if compared to a single step digestion protocol, and that using PPS Silent™ Surfactant in the second step potentiates the results.

Step 2 increases protein sequence coverage

1248 peptides were identified in Step 1. With PPS Silent™ Surfactant included, Step 2 resulted in the identification of 1428 peptides of which 743 were new when compared to Step 1, bringing the total to 1991. Without detergent, 936 peptides were identified of which 325 were new (Figure 1).

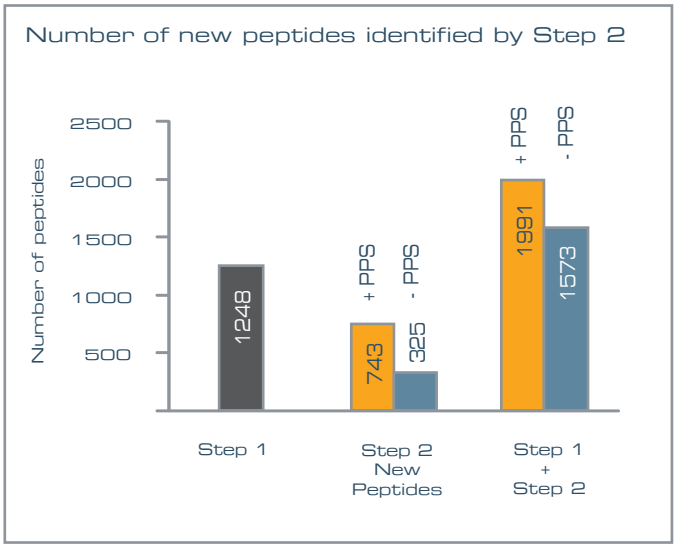

Figure 1. Step 2 increased the total number of peptide identifications by 325 without detergent, and by 743 with PPS Silent™ Surfactant.

The new peptides identified in Step 2 increased the sequence coverage of proteins identified in Step 1. Of the 446 proteins identified in Step 1 (including single-peptide identifications), the sequence coverage of 186 proteins was increased by Step 2 when PPS Silent™ Surfactant was used. Using PPS Silent™ Surfactant, it was possible to increase sequence coverage by up to 30% for some proteins. Without detergent in Step 2, the sequence coverage of 141 proteins was increased (Figure 2).

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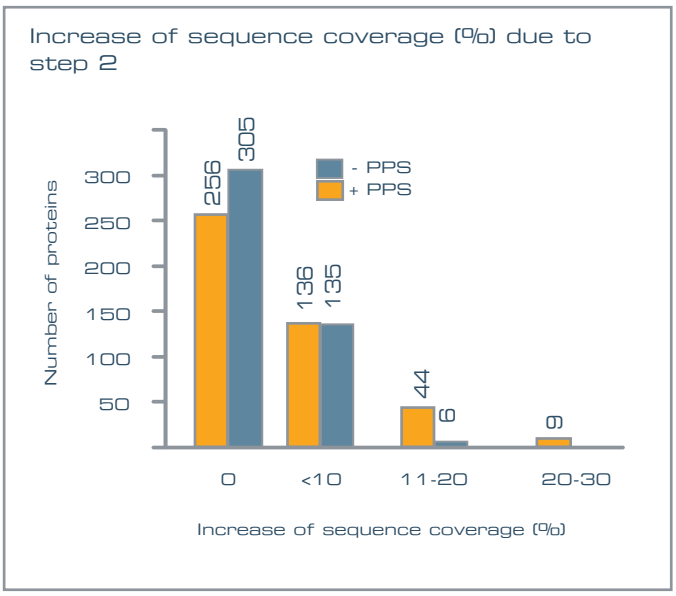

Figure 2. Step 2 increased sequence coverage of proteins identified in Step 1. PPS Silent™ Surfactant potentiated the sequence coverage increase.

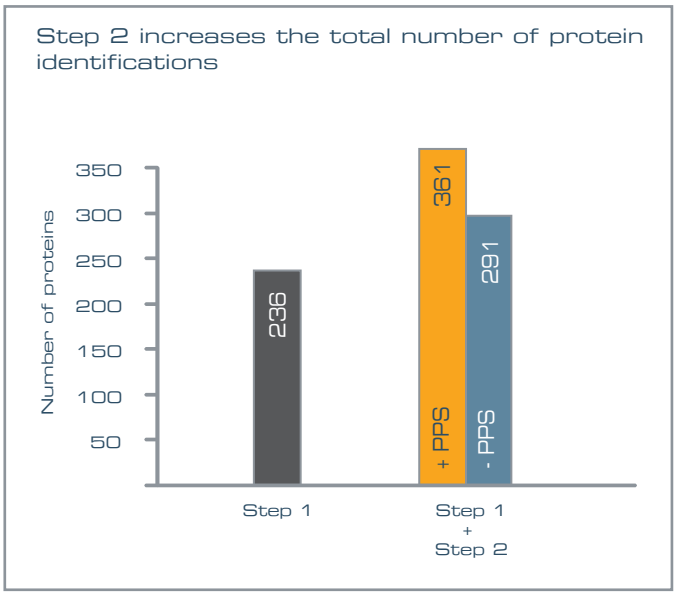

Figure 3. Step 2 increased the total number of confident protein identifications by 55 without detergent, and by 125 with PPS Silent™ Surfactant. Data is based on proteins identified by at least 2 peptide sequences.

Step 2 increases the number of confident protein identifications

236 confident protein identifications (at least two peptide sequences per protein) were made based on peptide data from Step 1. The data from Step 2 identified 63 additional proteins when PPS Silent™ Surfactant was used. In the absence of detergent, in Step 2, the number of additional proteins was 15. Step 2 also added complementary peptide sequences to proteins identified in Step 1. With PPS Silent™ Surfactant included, 62 proteins identified by a single peptide in Step 1 received at least another peptide sequence from Step 2. Without detergent the number of proteins that received at least one more peptide sequence was 40. In summary, the total increase in number of confident protein identifications due to Step 2 was 125 using PPS Silent™ Surfactant and 55 without (Figure 3).

Conclusions

The LPI™ technology enables immobilization of native membrane protein preparations and provides a tool for easily exchanging the solution environment around the membrane proteins. This allows for sequential digestion of the sample thus increasing the number of identified peptides. This approach results in several benefits compared to a single digestion step:

- Improved sequence coverage
- Increased confidence in protein identification
- More protein identifications

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