**ITSIPrep™ PROTEIN DIGESTION MONITORING (ProDM) KIT**


**BACKGROUND:** Protein identification by mass spectrometry is one of the most important steps in proteomics. Proteins are typically digested into peptides prior to the mass spectrometry (MS) step. The most popular enzyme used for protein digestion is trypsin. This enzyme is particularly suited for digestion because it has a very well defined specificity. Currently, MS and proteomics workflow processes do not include a step to verify that the trypsin is active and/or that the target protein has been adequately digested. Thus, tryptic digests are analyzed by MS without knowing whether the protein was digested or not.

**ITSIPrep Protein Digestion Monitoring (ProDM, K-0021-10) Kit (Patent pending)** is a distinctive product that allows precise determination of the % protein digested using a proprietary colorimetric reagent and any spectrophotometer. By using the protein standard provided in the kit, you are also able to simultaneously determine if your enzyme (e.g. trypsin, chymotrypsin) is active. ProDM kit eliminates the need to perform gel electrophoresis, to determine if your enzyme (e.g. trypsin, chymotrypsin) is active.

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**Solution Digestion Protocol for Standard and Unknown sample:**

1. Transfer 20ul of PS to a clean microfuge tube (provided).
2. Add 6ul of 10mM DTT to each tube. Mix by repeated pipetting.
3. Incubate at Room Temperature for 20 min.
4. Add 4ul of 50mM Iodoacetamide to each tube. Mix by repeated pipetting.
5. Incubate in the dark at Room Temperature for 15 min.
6. Add 140ul of RB to each tube. Mix by repeated pipetting.
7. Add 5ul of 0.2ug/ul sequencing grade trypsin or a suitable digestion enzyme to each tube. Mix by inversion.

**Determination of % Protein Digested:**

8. Immediately after adding enzyme (e.g. trypsin) and mixing, transfer 10ul of the reaction mixture (containing the target protein and enzyme) into tube labelled T0.
9. Add 5ul of RQ to tube T0 immediately.
10. Vortex immediately and spin briefly. Store at Room Temperature until analyzed on same day, or at -20°C for overnight storage.
11. At the end of your standard tryptic digestion process, transfer 10ul of the reacted mixture into the tube labelled Tx.
12. Repeat steps 9 and 10.
13. Set up the Blank (B) by transferring 10ul RB and 5ul of RQ to the tube labeled "B'.
14. Add 200ul of DR to the tube labeled T0, 200ul to the tube labelled Tx and 200ul to the tube labeled B.
15. Vortex and spin briefly, and incubate at room temperature for 5 min.
16. Use Distilled & De-ionized water to zero the spectrophotometer at 585nm.
17. Read the absorbance of B, T0 and Tx at 585nm. It is important that the spectrophotometer is blanked with water before reading the absorbance of B, T0 and Tx.
18. Calculate % Protein Digested (%PD) with the formula below:

\[
\%PD^* = \frac{(A_{T0} - B) - (A_{Tx} - B)}{A_{T0}} \times 100
\]

**Where,**

\[
A_{T0} = \text{Absorbance at } 585\text{nm for } T0
\]

\[
A_{Tx} = \text{Absorbance at } 585\text{nm for } Tx
\]

%PD of ≥75% is recommended for mass spectrometric analysis. T0 is Incubation Time zero and Tx is any Incubation Time greater than zero.

**Conditions for use of this procedure/Buffers:**

This protocol is the intellectual property of ITSI Biosciences. Only complete set of reagents provided by ITSI Biosciences should be used. Considering that many factors can cause experiments to fail, ITSI Biosciences cannot guarantee that the use of this protocol and reagents will lead to a successful experiment. In no event shall ITSI Biosciences be held liable for loss of samples, failure of experiments or any other damage or injury associated with the use of this product.

**General Safety Information and conditions for using the product:**

Consider all chemicals as potentially hazardous. Only trained laboratory personnel familiar with good laboratory practice should handle this product. Protective clothing and gloves should be worn at all times. Use caution to avoid contact with skin and eyes. If contact should occur, wash immediately with plenty of water and follow established guidelines/procedures in your laboratory. Warning: The procedure and kit are intended for research use only, not for use in human, therapeutic, or diagnostic applications. While ITSI will replace defective products, it does not accept any responsibilities for improper use of this product, or loss/damages to samples. The end user is responsible for all local, state and federal regulations associated with the use and disposal of laboratory reagents.