



ITSIPrep™ PROTEIN DIGESTION MONITORING KIT(ProDM)

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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 using trypsin. 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 completely 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. ProDM kit eliminates the need to perform gel electrophoresis, to confirm that the enzyme is active and the protein is adequately digested. ProDM will reduce the number of failed mass spectrometry experiments attributable to inactive enzyme and/or inadequate protein digestion.

Read the procedure completely and assemble all materials needed before starting.

MATERIALS PROVIDED IN THIS KIT (Sufficient for 10 Assays):

ITEM	QTY	CAT. #	STORE*
Reaction Quencher (RQ)	1 x 0.5 ml	K-0021-01	4° C
Detection Reagent (DR)	1 x 30 ml	K-0021-02	4° C
Reaction Buffer (RB)	1 x 5 ml	K-0021-03	-20° C
Procedure	1	VP0021-10	

*Shipped at Room Temperature, but requires different storage.

MATERIALS REQUIRED BUT NOT SUPPLIED:

- Sequencing grade trypsin or a different enzyme e.g. chymotrypsin.
- 10mM dithiothreitol (DTT) or another reducing agent e.g. Tris (2-carboxyethyl) phosphine (TCEP).
- 50mM Iodoacetamide (IAA).
- Deionized water.
- Vortex mixer.
- Adjustable pipettes.
- Spectrophotometer capable of reading wavelengths between 570nm and 610nm.
- Microcentrifuge tubes

PROCEDURE:

Determination of % Protein Digested:

- Vortex and spin **DR** briefly before use.
- Label tubes *To* (Time 0) and *Tx*. (Time *x*).
- Immediately after adding enzyme (e.g. trypsin) to your protein sample and mixing, transfer 10 µl of the reaction mixture (containing the target protein and enzyme) into tubes labelled *To*.
- Add 5 µl of **RQ** to tubes labeled *To* immediately.
- Vortex immediately and spin briefly. Store on ice for same day analysis, or at -20°C for overnight storage.
- At the end of your standard tryptic digestion process, transfer 10 µl of the digested sample(s) into the tubes labeled *Tx*.
- Repeat steps 4 and 5.
- Set up the Blank (B) by transferring 10 µl **RB** and 5 µl of **RQ** to a tube labeled B.

- Add 1ml of **DR** to the tube(s) labeled *To*, 1ml to the tube(s) labelled *Tx* for your sample(s) and 1ml to the tube labeled B.
- Vortex and spin briefly, and incubate at room temperature for 5 min.
- Use deionized water to zero the spectrophotometer at 585nm.
- Read the absorbance of B, *To*, and *Tx* at 585nm.
- Calculate % Protein Digested (%PD) for your sample with the formula below:

$$\%PD^* = \text{Change in Absorbance at } 585\text{nm}(\Delta A_{585\text{nm}}) \times 100$$

Where,

$$\Delta A_{585\text{nm}} = \frac{(A_{T_0} - B) - (A_{T_x} - B)}{A_{T_0} - B}$$

*% PD of ≥75% is recommended for mass spectrometric analysis. *T*₀ is Incubation Time zero and *T*_x is any Incubation Time greater than zero.

N/B: *A*_{*T*_x} < *A*_{*T*₀} indicates that digestion has occurred. If *A*_{*T*_x} is not < *A*_{*T*₀} for the experimental samples, then enzyme is likely inactive.

*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.

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.**