ITSI BIOSCIENCES

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 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, and hence the number of failed experiments. **Read the procedure completely and assemble all materials needed before starting**.

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

ITEM	QTY	CAT. #	STORE
Protein Standard (PS)	1 x 500 mg	K-0021-01	4°C#*
Standard Buffer (SB)	1 x 1 ml	K-0021-02	-20°C#
Reaction Buffer (RB)	1 x 2 ml	K-0021-03	4°C#*
Reaction Quencher (RQ)	1 x 0.5 ml	K-0021-04	4° C#
Detection Reagent (DR)	1 x 8.0 ml	K-0021-05	4° C#
Microfuge tubes	30 x 0.5 ml	K-0021-06	RT
Procedure	1	VBP0021-10	

#Shipped at Room Temperature (RT), *Store at –20°C.

MATERIALS REQUIRED BUT NOT SUPPLIED:

i. 0.2ug/ul sequencing grade trypsin or an appropriate concentration of a different enzyme e.g. chymotrypsin.
ii. 10mM ditiotreitol (DTT) or another reducing agent like Tris (2-carboxyethyl) phosphine (TCEP).
iii. 500 mM Iodoacetamide.
iv. Vortex mixer.
v. Adjustable pipettes.
vi. Spectrophotometer capable of reading wavelengths between 570nm and 610nm.
vii. Wet ice.

BEFORE STARTING:

i. Add 1ml of **SB** to **PS** to obtain 0.5g/ml of a protein standard stock solution. Mix properly by repeated inversion. **USE THIS IN ALL STEPS THAT REQUIRE PS**. Aliquot and Store reconstituted **PS** at –20°C to avoid repeated freeze-thaw cycles of the same tube. Bring **PS** out of the freezer (if stored at –20°C) and bring to room temperature before use.
ii. Vortex and spin **PS** and **DR** briefly. Place **PS** on ice.
iii. Label one microfuge tube *T0* (Time 0) and a second *Tx* (Time X). Label a third tube Blank (B). Label the appropriate number of tubes if working with more than one sample. E.g. *T01*, *To2* and *Tx1*, *Tx2* if working with two samples.

PROCEDURE:

Solution Digestion Protocol for Standard and Unknown sample:

1. Transfer 20ul of **PS** to a clean microfuge tube (provided). **Also transfer 20ul of your sample to a second tube.**
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 pipeting.
5. Incubate in the dark at Room Temperature for 15 min.
6. Add 140ul of **RB** to each tube. Mix by repeated pipeting.
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 *To*.
9. Add 5ul of **RQ** to tube *To* 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 *To*, 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, *T*0 and *T*x.**
18. Calculate % Protein Digested (%PD) with the formula below:

%PD* = Change in Absorbance at 585nm (ΔA585nm) x 100

Where,

$$\Delta A_{585nm} = \frac{(AT_0 - B) - (AT_x - B)}{AT_0 - B}$$

*% 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: $AT_x < AT_0$ indicates that digestion has occurred. If AT_x is not $< AT_0$ for the standard and experimental samples, then enzyme is likely inactive. If AT_x is $< AT_0$ only for the standard protein sample, then the enzyme is likely active, but not suitable for digesting the experimental protein.

***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.**

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