ProteoBind On-column Proteolytic Digestion Kit

Product Code:

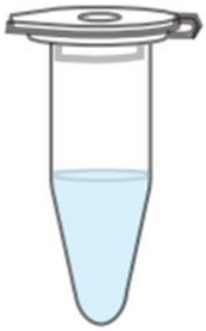
BIO-5300-10
BIO-5300-50

Ver. 1.0

Kit Content

No.	Buffer	Quantity		Storage Condition
		10 Reactions	50 Reactions	
1	Denaturant	1 x 125µL	1 x 625µL	20 – 25 °C
2	Buffering agent	1 x 25µL	1 x 125µL	Store at 2 – 8 °C upon receipt.
3	Reducing agent	1 x 10µL	1 x 50µL	
4	Alkylating agent	3mg in 64.2µL of water	12mg in 256.8µL of water	20 – 25 °C
5	Acidifier	1 x 50µL	1 x 250µL	
6	Protein precipitating solution	1 x 15.5ml	1 x 77.5ml	
7	Digestion buffer	2 x 1ml	1 x 10ml	
8	Elution buffer 1	1 x 1ml	1 x 5ml	
9	Elution buffer 2	1 x 1ml	1 x 5ml	

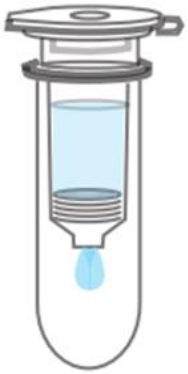
ON-COLUMN PROTEIN DIGESTION QUICK GUIDE



1-step Denaturation, Reduction, Alkylation, Acidification and Precipitation

Step 1: Prepare 100µg of proteins in 1.5ml tube. Max sample volume: 30µl
Step 2: Denaturation (12.5µl of Buffer-1, Tube ①). Mix well.
Step 3: Buffering (2.5µl of Buffer-2, Tube ②). Mix well.
Step 4: Reduction (1.0µl of Buffer-3, Tube ③). Mix well.
Step 5: Alkylation (4.0µl of Buffer-4, Tube ④). Mix well.
Step 6: If needed, top-up with nuclease-free water. Final volume: 50µl.
Step 7: Mix well. Incubate mixture at 55°C for 10mins.
Step 8: Acidification (5µl of Buffer-5, Tube ⑤). Mix well.
Step 9: Protein precipitation (350µl of Buffer-6, Tube ⑥). Mix well.

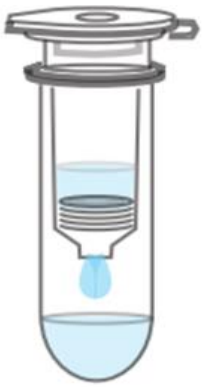
⇩



On-column Protein-binding and Washing (Discard the flow-through)

Step 10: Transfer the entire mixture (including any particulate) to spin column.
Step 11: Centrifuge at 4000 x g, 25°C for 30 secs.
Step 12: Collect flow-through and repeat Steps 10 and 11, on the same column.
Step 13: Discard the flow-through after second protein-binding.
Step 14: On-column washing (400µl of Buffer-6, Tube ⑥).
Step 15: Centrifuge at 4000 x g, 25°C for 30 secs.
Step 16: Repeat Steps 14 and 15, two more times. Total: 3 washes.
Step 17: Centrifuge at 4000 x g, 25°C for 1 min, to remove residual of Buffer-6.

⇩



On-column Digestion and Elution (Keep all the flow-through)

Step 18: Transfer to spin column into a clean collection tube.
Step 19: Digestion (100µl of Buffer-7, Tube ⑦).
Step 20: Centrifuge at 4000 x g, 25°C for 15 secs.
Step 21: Reload the flow-through onto the same column.
Step 22: Incubate for 1 hr at 37°C.
Step 23: Add 100µl of Buffer-7, Tube ⑦. Centrifuge at 4000 x g, 25°C, 1 min.
Step 24: Add 100µl of Buffer-8, Tube ⑧. Centrifuge at 4000 x g, 25°C, 1 min.
Step 25: Add 100µl of Buffer-9, Tube ⑨. Centrifuge at 4000 x g, 25°C, 1 min.
Step 26: Add 100µl of Nuclease-free water to the pooled eluates.
Step 27: Transfer the pooled eluates to pre-labelled 1.5ml tube.
Step 28: Sample is ready for analysis, or be kept at -80°C.

PRODUCT MANUAL

Introduction

This protocol describes the detailed procedures for an on-column protein digestion on general protein samples, such as cell lysates, protein extracts and purified proteins. The streamlined workflow and optimized reagents enable complete protein digestion with high peptides recovery rate, leading to a rapid, highly reproducible, and versatile protein digestion at high confidence. The simple spin procedure, which is ideal for simultaneous processing of multiple samples, yield protein digest ready for mass spectrometry (MS) analysis in just 70 minutes.

Intended Use

This kit is intended for mass spectrometry-ready protein digestion. This product is not intended for the diagnosis, prevention, or treatment of a disease. All due care and attention should be exercised in the handling of the products. We recommend all users to adhere to the NIH guidelines that have been developed or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs).

Amount of Starting Material

The optimized protocol enables complete digestion of 100µg protein (~30µl of protein at 3.4mg/ml). If sample concentration is higher than 3.4mg/ml, scale down the sample volume accordingly to obtain a 100µg of protein per digestion. For protein samples with concentration lower than 3.4mg/ml, it is recommended to concentrate the sample using SpeedVac or 30,000 MWCO centrifuge spin column prior to protein digestion.

Matters to Note Before Starting

1. Pre-heat the water bath (37°C) and heat block (55°C)
2. Reconstitute Buffer-4 with 64.2µl of Nuclease-free water. Mix well before use.
3. Appropriate amount of trypsin (not provided) is added to Buffer-7 before use. In general, 4µg of trypsin is required for 100µg of protein digestion.

Protocol

Sample preparation, denaturation, reduction, alkylation, acidification, and precipitation.

1. Prepare 100µg of protein per digestion, in a 1.5ml microcentrifuge tube (not provided). For more information, refer to "Amount of Starting Material" section.
2. For denaturation, add **12.5µl of Buffer-1 (Tube ①)** to each sample. Mix well through gentle pipetting.
3. Add **2.5µl of Buffer-2 (Tube ②)** to each sample to buffer the mixture. Mix well through gentle pipetting.
4. For reduction, add **1µl of Buffer-3 (Tube ③)** to each sample. Mix well through gentle pipetting.
5. For alkylation, add **4µl of Buffer-4 (Tube ④)** to each sample. Mix well through gentle pipetting.
6. If necessary, top-up the reaction volume with nuclease-free water (NFW) to a final volume of 50µl. See Table 1 for the calculation and digestion of 100µg protein sample.

Table 1: Calculation for 100µg protein reaction

Sample concentration, in mg/ml	Volume required, in µl						Total volume, in µl
	Sample volume	Buffer-1	Buffer-2	Buffer-3	Buffer-4	NFW	
X	100/X	12.5	2.5	1.0	4.0	30-(100/X)	50
3.4	29.4	12.5	2.5	1.0	4.0	0.6	50

7. Briefly vortex the sample tube to mix, spin down the content, and incubate the sample tube on pre-heated heat block at 55°C for 10 minutes.
8. After 10-minute incubation, add **5µl of Buffer-5 (Tube ⑤)** to each sample tube. Vortex to mix well, and briefly spin down the content.
9. Add **350µl of Buffer-6 (Tube ⑥)** to each sample tube. Mix through gentle pipetting.

   Note: The appearance of mixture may turn milky due to the formation of colloidal protein particulate.

On-column protein-binding

10. Assemble the spin column accordingly. Transfer the entire volume of the mixture, including any protein particulate, onto the assembled spin column, and centrifuge at 4000 *x g* at 25°C for 30 seconds.
11. Collect the flow-through and reload onto the same spin column to maximize protein binding.
12. Repeat the centrifugation and discard the flow-through after the second protein-binding.

On-column washing and digestion

13. To wash the captured proteins, add **400µl of Buffer-6 (Tube ⑥)** to each sample and centrifuge at 4000 *x g* at 25°C for 30 seconds. Discard the flow-through.
14. Repeat Step 13 two more times. Total number of washes: 3.
15. Centrifuge again at 4000 *x g* at 25°C for 1 minute to remove residual of Buffer-6.
16. Transfer the spin column onto a new collection tube.
17. Add **100µl of Buffer-7 (Tube ⑦)** onto membrane of each spin column. Let the solution flow through the spin column by centrifugation at 4000 *x g* at 25°C for 15 seconds.

    Note-1: Ensure that Buffer-7 is supplemented with appropriate amount of trypsin (not provided). For 100µg of protein digestion, 4µg of trypsin is needed.

18. Collect and reload the flow-through onto the same membrane of spin column. Gently cap the spin column and incubate the whole spin column together with the collection tube in pre-heated water bath at 37°C for 1 hour.

    Note-1: It is important to cap the spin column gently. Forceful-capping of the spin column may cause leaking of Buffer-7 from the membrane, leading to lower digestion efficiency and hence yield.
    Note-2: Do not shake and do not introduce bubbles onto the membrane. Avoid bubbles in Buffer-7.
    Note-3: Ensure the entire membrane is exposed to heat. To do this, make sure the entire membrane is below the water level.
    Note-4: Do not wrap the spin column during digestion at 37°C. An airtight seal will cause Buffer-7 being forced through the membrane into the collection tube. See Note-1.

Elution

19. After 1-hour incubation at 37°C, add **100µl of Buffer-7 (Tube ⑦)** onto each membrane of the spin column. Centrifuge the spin column (with the collection tube) at 4000 *x g* at 25°C for 1 minute.

    Note: Do NOT discard the flow-through.
20. Add **100µl of Buffer-8 (Tube ⑧)** onto each membrane of the spin column. Centrifuge the spin column (with the same collection tube containing flow-through from Step 19) at 4000 *x g* at 25°C for 1 minute.

    Note: Do NOT discard the flow-through.
21. Add **100µl of Buffer-9 (Tube ⑨)** onto each membrane of the spin column. Centrifuge the spin column (with the same collection tube containing flow-through from Steps 19 and 20) at 4000 *x g* at 25°C for 1 minute.

    Note: Do NOT discard the flow-through.
22. Collect the pooled flow-through (from Steps 19, 20 and 21) and transfer to a new pre-labelled 1.5ml microcentrifuge tube (not provided).
23. Add **100µl of nuclease-free water (not provided)** into the pooled eluate (from Step 22) to produce a final protein digest at ~0.2µg/µl peptides.
24. The final protein digest is now ready for analysis. Alternatively, it can be kept in -80°C.