Total Protein Extraction Kit for Adipose Tissues/Cultured Adipocytes

Cat. #: P522-4 (4 reactions); P522-20 (20 reactions); P522-80 (80 reactions)

Storage: Store Buffer A at 4°C, and the rest of the kit at room temperature

Shelf Life: 12 months

Product Description:

Fast extract total protein from white and brown adipose tissue (WAT/BAT), and cultured adipocytes in 20 minutes with high yield (2~3 mg/ml).

This product is for research use only.

Product Contents

Component	Amount			Storage
	Cat.#: P522-4	Cat.#: P522-20	Cat.#: P522-80	
Product size	4 reactions	20 reactions	80 reactions	
Buffer A (extraction buffer)	3 ml	15 ml	60 ml	4°C
Buffer B (10X denaturing buffer)	0.3 ml	1.5 ml	6 ml	Room temperature
Buffer C (10X non-denaturing buffer)	0.3 ml	1.5 ml	6 ml	Room temperature
Microfuge tube (1.5 ml)	4	20	80	Room temperature
Pestles for 1.5 ml tube	1	2	8	Room temperature
Protein extraction filter cartridges with collection tubes	4	20	80	Room temperature
Protein extraction powder	0.5 g	2 g	8 g	Room temperature

Additional Materials Required

- Table-Top Microcentrifuge with a maximum speed of 14,000-16,000 rpm.

Notes:

1. Protease inhibitors is not necessary prior to extraction. However if downstream application takes significant amounts of time or the protein extract will be stored for longer period of time, addition of protease inhibitors to Buffer A is recommended.
2. For determination of protein concentration, BCA kit (Pierce) is recommended.
3. To study protein phosphorylation, phosphatase inhibitors (such as PhosStop from Roche) should be added to Buffer A prior to use.

*If precipitate is found in Buffer B at lower temperature, incubate at >37°C until the precipitate is completely dissolved.

Protocol:

First of all, pre-chill Buffer A and filter cartridges in collection tubes on ice.

A. Protein Extraction from Adipose Tissues (WAT or BAT)

1. Weight **50-80 mg** fresh or frozen adipose tissue, place it between a few layers of paper towel and squeeze with thumb and index finger to remove a portion of oil from the tissue. Use forceps to place the tissue in the bottom of a 1.5 ml microfuge tube provided (don't use other 1.5 ml tube from your Lab because it may not fit the pestle provided). Weigh **100 mg protein extraction powder** and add to the tube on top of the tissue. Add **50 µl** Buffer A to the tube.

2. Grind the tissue with a pestle with twisting force for about 1-2 min to reduce the tissue to slurry. Add 200-300 µl Buffer A to the tube and continue to grind for another 30 seconds. If small amount of starting tissue (20-40 mg) is used add 100-150 µl Buffer A to the tube.

3. Cap the tube and centrifuge at 2,000 rpm for 1 min. Transfer supernatant to a filter cartridge with collection tube (it does not matter if some fat aggregate is carried over).

Note: The pestle is reusable: after use, wipe it with 75% alcohol and air dry.

4. Incubate the filter cartridge with cap open at -20°C for 20 min. Check the temperature of your refrigerator to make sure that the temperature is around -20°C. Otherwise refer to trouble shooting below.

5. After incubation immediately centrifuge at 2,000 rpm for 2 min with cap open. The flow through contains total proteins from adipose tissue. Extracted proteins appear slightly clouded due to the presence of water insoluble cellular components. It can be diluted and directly use in ELISA for detection of water soluble proteins. It can also be re-suspended in buffer B or buffer C to dissolve water insoluble proteins for downstream applications:
   A. Add 1/10 of Buffer B to the extracted protein solution resulting in a denatured protein solution (Ideal for SDS-PAGE, Westerns and other applications) or
   B. Add 1/10 of Buffer C to the extracted protein solution resulting in a non-denatured protein solution (ideal for IP, ELISA and other applications) or
   C. Dissolve in 2 X 2D gel sample buffer for 2D gel analysis.

Note: Buffer A contains a component that may interfere with mass spec analysis. If extracted proteins are used for MS analysis, dialysis against compatible buffer first. Another option is to precipitate proteins using standard TCA protein precipitation protocol.

B. Protein Extraction from Cultured Adipocytes

1. Harvest 50-100 million cultured adipocyte by low speed centrifugation. Resuspend the cells in a 1.5 ml tube with **1 ml cold PBS**. Add **100 mg protein extraction powder** to the tube.

2. Centrifuge in a microfuge at 3,000 rpm for 3 min. Remove supernatant completely. Grind the cells with pestle with twisting force for about 1-2 min to homogenize the cells. Add **200-300 µl** Buffer A to the tube and continue to homogenate for another 30 seconds.

3. Centrifuge at 2,000 rpm for 1 min and transfer the supernatant to a pre-chilled filter cartridge with collection tube.

4. Incubate the filter cartridge **with cap open at -20°C for 20 min**. Check the temperature of your refrigerator to make sure that the temperature is around -20°C. Otherwise refer to trouble shooting below.

5. After incubation immediately centrifuge at 2,000 rpm for 2 min with cap open. The flow through contains total proteins from adipose tissue. Extracted proteins appear slightly clouded due to the presence of water insoluble cellular components. It can be diluted and directly use in ELISA for detection of water soluble proteins. It can also be re-suspended in buffer B or buffer C to dissolve water insoluble proteins for downstream applications:

   D. Add 1/10 of Buffer B to the extracted protein solution resulting in a denatured protein solution (Ideal for SDS-PAGE, Westerns and other applications) or

   E. Add 1/10 of Buffer C to the extracted protein solution resulting in a non-denatured protein solution (ideal for IP, ELISA and other applications) or

   F. Dissolve in 2 X 2D gel sample buffer for 2D gel analysis.

Troubleshooting

The incubation time of **step 5** is critical for clear separation of aqueous phase from oil phase in the tissue homogenate. There are variations in the actual temperature of the refrigerator in a particular lab. We recommend to perform a simple test to determine the optimal incubation time:

Add 0.5 ml ddH_2O to a normal **1.5 ml microfuge tube used in your lab** and incubate in your refrigerator with the cap open. Determine minimum time required to freeze the water completely. This is the optimal incubation time for **step 5**. You can also perform this test in a -80°C freezer. The use of -80°C freezer will significantly reduce the incubation time in step 5.

Remarks: This protocol is developed and validated by 101Bio's OEM partner. Spin column based protein extraction and cell. fractionation technologies were developed by 101Bio's OEM partner.

Related products

Cat.#	Kit Name	Application			Protein Status	min.
P501	Total protein kit	cells	→	Total protein	Denatured / Native	1 ~ 8
P502	Total protein kit	tissues	→	Total protein	Denatured / Native	1 ~ 8
P503	Membrane protein kit	cells / tissues	→	Membrane and cytosol protein	Native & Detergent-free	20 ~ 45
P504	Nuclear protein kit	cells / tissues	→	Nuclear & cytosol protein	Native	6 ~ 8
P505	Detergent-free kit	cells	→	Total protein	Denatured / Native	5 ~ 8
P506	Detergent-free kit	Tissues	→	Total protein	Denatured / Native	5 ~ 8
P507	Mitochondria kit	cells / tissues	→	Mitochondria	Native & Detergent-free	25 ~ 30
P508	Plant total protein	plant tissues	→	Total protein	Denatured/Native	5 ~ 8
P510	Plant detergent-free	plant tissues	→	Total protein	Native	6 ~ 8
P511	Plant chloroplast kit	plant tissues	→	Intact chloroplast		5
P512	Bacteria total protein	bacteria	→	Total protein	Denatured	2 ~ 3
P513	Nuclear envelope kit	Cells	→	Nuclear envelope	Native	< 45
P514	Histone/DNA binding protein extraction kit	Cells	→	Histone & DNA binding protein	Denatured	< 10
P515	Thick cell wall microbes protein kit	Microbes	→	Total protein	Denatured / Native	< 10
P519	Gel slice recovery kit	PAGE gel	→	Protein	Denatured / Native	10 ~ 20
P521	Hair & nail protein kit	Hair, nail	→	Protein	Denatured	5 min. hands on
P522	Adipose protein kit	Adipose / adipocyte	→	Total Protein	Denatured / Native	20

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