Minute™ Total Protein Extraction Kit for Insects

Catalog number: SA-07-IS

Description

Despite of significant variation in body organization, insects all have the same general body structure. They have segmented bodies divided into three regions: head, thorax and abdomen. The body segments are protected by a hard exoskeleton or cuticles. From protein extraction point of view, the unique structure of exoskeleton makes it very hard to homogenize. It is also very difficult to lyse cells protected by cuticle for total protein extraction. The traditional solution-based protein extraction method such as RIPA is inefficient and protein yield is low. The profile of extracted protein using traditional method is usually incomplete. This kit provides a highly efficient method for total protein extraction from insects by a combination of mechanical extraction and chemical lysis. The cell lysis buffers used are much stronger than RIPA buffer. The kit features a simple and rapid single tube protocol and optimized buffers for insect tissues. The researchers have the option to choose either denaturing or native cell lysis buffer, which are specifically tailored for insects. The whole procedure takes less than 10 min to complete and the protein yield is in the range of 1-3 mg/ml. The materials provided are sufficient for 50 extractions.

Applications

Proteins extracted with this kit can be used for many downstream applications such as SDS-PAGE analysis, Western blotting, IP. ELISA, enzyme activity assays and proteomic analysis. The buffers are compatible with IMAC resins for his-tagged protein purification. The salts and detergents in the extracted protein sample should be removed prior to mass spectrometry analysis

Kit components

1.	Denaturing Buffer	25 ml
2.	Native Buffer	25 ml
3.	Protein Extraction Powder	5g
4.	Plastic Rod	2
5.	Filter Cartridge	50
6.	Collection Tube	50

Shipping and storage: This kit is shipped and stored at room temperature

Additional Materials Required

Table-Top Microcentrifuge with a maximum speed of 14,000-16,000 X g

Important Product Information

Denaturing buffer contains ionic detergent and other chemicals for solubilization of proteins. It may form precipitate at low temperature. It is not recommended to pre-chill it

Invent Biotechnologies, Inc., 1864 Berkshire Ln N, Plymouth, MN 55441
Tel: (952)400-6898, Fax: 952-400-6889, info@inventbiotech.ocm, www.inventbiotech.com

on ice. Native buffer can be pre-chilled and will not form precipitate. The lysis buffers do not contain protease inhibitors. If proteolysis is a concern, it is recommended to add protease inhibitor cocktails to aliquot of the buffers prior to use. For determination of protein concentration, BCA kit (Pierce) is recommended. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) must be added to the buffer prior to use.

Protocol

For demonstration purpose, following amount of starting material and lysis buffer are recommended However, the protocol can be scaled up or down proportionately. The protocol can be performed at ambient temperature.

1. Weight out 20-30 mg tissue (fresh/frozen) and cut it with a sharp blade or a pair of scissors into small pieces (1 X 1 mm or smaller). Place cut tissue in a filter cartridge with collection tube. For small insects such as drosophila and mosquitos, 10 to 20 whole insects or larvae can be placed in the filter without cutting.

2. Add 50 to 80 mg protein extraction powder on top of the tissue followed by addition of 100 ul lysis buffer.

3. Immediately grind the tissue with the plastic rod provided against the surface of the filter with moderate twisting force for 2-3 min. Add another 100 ul lysis buffer to the filter and continue to grind for about 30 seconds to 1 min. **The plastic rod is reusable. Clean it with 70% alcohol or water.**

4. Centrifuge the tube at top speed in a microcentrifuge for 1 min. Remove and discard the filter. Transfer the supernatant to a fresh tube (this is extracted total protein). The white-grey pellet in the bottom of collection tube is passing through protein extraction powder that should be discarded.

Application tips: If the final protein yield is low, incubate grinded tissue in step 3 at room temperature for 5-10 min or increase grinding time. During incubation period, the lysis buffer may drip into collection tube. This is normal and will not affect the quality of extracted protein.

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