## Brief Protocol

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|---|---|
| Homogenization | Homogenize up to 2 x $10^7$ of cultured animal cells or 100 mg of animal tissue in 1 ml of ProtinEx™ Animal cell / tissue. |
| Incubation | Incubate for 5 minutes on ice. |
| Debris precipitation | Centrifuge at 16,000 x g for 10 minutes at 4°C. |
| Protein separation & Use | Transfer the supernatant to a fresh tube.<br>Supernatant can be directly used for downstream applications. |

## Troubleshooting Guide

| Problem | Possible cause | Suggested solution |
|---|---|---|
| Low yield of Protein | Low Sample mass | Check the sample mass. |
| | Insufficient amount of ProtinEx™ Animal cell / tissue was used | Add more ProtinEx™ Animal cell / tissue. |
| | Sample not homogenized completely | Make sure no particulate matter remains. Incubate optionally for 5 minutes on ice after homogenization. |
| | Protein expression was low | Check the transfection efficiency. |
| Degraded Protein | Exposed sample for long time in a room temperature | Do not keep too long time in a room temperature while doing lysis with ProtinEx™ Animal cell / tissue. |
| | Sample manipulated too much before the addition of ProtinEx™ Animal cell / tissue | Until the treatment of ProtinEx™ Animal cell / tissue, sample should be kept on the ice. |
| | Working of endogenous protease | Use a protease inhibitors cocktail.<br>Incubate on ice. Confirm the absence of the protease contamination on the equipment. |



GeneAll®

*For research use only*

# ProtinEx™

## Animal cell / tissue

**Cat. No. 701-001**

**Size: 100 ml**

## Kit Contents (for 100 preps)

| Components | Quantity | Storage |
|---|---|---|
| ProtinEx™ Animal cell / tissue | 100 ml | Store at 4°C |

## Quality Control

ProtinEx™ Animal cell / tissue is manufactured in strictly clean condition and its degree of cleanness is monitored periodically. For consistency of product, the quality certification process is carried out thoroughly and only the qualified is delivered.

## Storage Conditions

ProtinEx™ Animal cell / tissue should be stored at 4°C and kept out of direct sunlight. ProtinEx™ Animal cell / tissue is stable at 4°C until the date of expiration that is printed on the product label.

## Precautions

When working with ProtinEx™ Animal cell / tissue, wear gloves and eye protector, keep out of contact with skin or clothing, and avoid breathing of vapor. In case of contact, wash immediately with plenty of water and seek medical advice.

## Materials Not Provided

- Microcentrifuge tubes, tips
- Cell scraper
- Equipment for homogenizing solid tissue
- Microcentrifuge
- Ice-cold PBS

## Product Disclaimer

GeneAll® ProtinEx™ Animal cell / tissue is for research use only, and should not be used for clinical or diagnostic purpose.

## Product Description

ProtinEx™ Animal cell / tissue provides fast and easy methods for the extraction of total soluble proteins from animal cells and tissues. When extracting proteins, efficient disrupting of cells or tissues is essential for recovering whole cellular proteins. Using ProtinEx™ Animal cell / tissue's optimized procedure, the cell membranes composed of phospholipids and membrane proteins can be easily and efficiently disrupted without further treatment like sonication or freeze / thaw step.

Owing to lack of ionic disturbance the denaturing power of non-ionic detergent is generally milder than that of ionic detergent. Non-ionic lytic condition of ProtinEx™ Animal cell / tissue enables the isolation of functionally active proteins which can be applied to protein-protein interaction experiments, reporter assays, protein assays, immunoassays, and protein purification.

ProtinEx™ Animal cell / tissue is designed to simplify and expedite the procedure of protein extraction. The sample harvested in ProtinEx™ Animal cell / tissue goes to incubating on ice for 5 minutes and centrifuging for 10 minutes to separate cell debris. The supernatant can be directly used for downstream applications, and the whole procedure takes only 30 minutes.

ProtinEx™ Animal cell / tissue procedure supports the extraction of proteins from up to 100 mg of animal tissues or 2 x $10^7$ of animal cells per one extraction. The maximum yield reaches 11 mg per 50 mg of animal tissues and 1300 ug per 1 x $10^7$ of animal cells, respectively.

## Protocol for Suspension-cultured animal cells

- The volumes given in this protocol are suitable for processing of 0.5 ~ 2 x $10^7$ animal cells. When processing larger or smaller cultures, adjust the volume of buffer used accordingly.
- ProtinEx™ Animal cell / tissue does not contain protease inhibitors.

**1. Pellet suspension-cultured animal cells by centrifugation at 2500 x g for 3 minutes. Discard (aspirate) the culture medium.**
Phenol red or other reagents in the culture medium interfere with protein analysis.

**2. Wash the pellet once with 10 ml of Ice-cold PBS by gently pipetting.**

**3. Pellet the suspension-cultured animal cells by centrifugation at 2500 x g for 3 minutes. Discard (aspirate) the Ice-cold PBS.**

**4. Add the 100 ul of ProtinEx™ Animal cell / tissue to the tube. Suspending the cell pellet by gently pipetting and transfer the suspending sample to a microcentrifuge tube (Not provided).**

**5. Add the 900 ul of ProtinEx™ Animal cell / tissue to the tube. Pipette the mixture up and down to complete lysis of the sample.**

**6. Incubate for 5 minutes on ice.**

**7. Centrifuge at 16,000 x g for 10 minutes at 4°C to remove the cell debris and transfer the supernatant to a fresh tube (Not provided).**

**8. The supernatant can be directly used for downstream applications.**
The supernatant contains the total protein fraction.

## Protocol for Monolayer-cultured animal cells

- The volumes given in this protocol are suitable for processing of 0.5 ~ 2 x $10^7$ animal cells. When processing larger or smaller cultures, adjust the volume of buffer used accordingly.
- ProtinEx™ Animal cell / tissue does not contain protease inhibitors.

**1. Decant (aspirate) the culture medium from 10 cm culture plate.**

**2. Wash the cells once with 10 ml of Ice-cold PBS by gently shake the plate for several times.**
Phenol red or other reagents in the culture medium interfere with protein analysis.

**3. Decant (aspirate) the Ice-cold PBS from culture plate.**

**4. Repeat Step 2 ~ 3.**

**5. Add the 1 ml of ProtinEx™ Animal cell / tissue to the plate. Incubate for 5 minutes on ice.**

**6. Collect the lysate by scrapper and transfer to a microcentrifuge tube (Not provided).**

**7. Centrifuge at 16,000 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube (Not provided).**

**8. The supernatant can be directly used for downstream applications.**
The supernatant contains the total protein fraction.

## Protocol for Animal tissue

- The volumes given in this protocol are suitable for processing of 50 ~ 100 mg animal tissue. When processing larger or smaller tissue, adjust the volume of buffer used accordingly.
- ProtinEx™ Animal cell / tissue does not contain protease inhibitors.

**1. Homogenize up to 100 mg of animal tissue in 1 ml of ProtinEx™ Animal cell / tissue by equipment for homogenizing solid tissue.**

**2. Incubate for 5 minutes on ice.**

**3. Centrifuge at 16,000 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube (Not provided).**

**4. The supernatant can be directly used for downstream applications.**
The supernatant contains the total protein fraction.

## The protein yields using the ProtinEx™ Animal cell / tissue

(Quantitative analysis of Protein by BCA assay)

| Cell lines | Amount of Starting material | Average yield of Total Protein |
|---|---|---|
| CHO | ≒ 1 x $10^7$ | ~ 319 ug |
| RAW264.7 | ≒ 1 x $10^7$ | ~ 500 ug |
| Jurkat | ≒ 1 x $10^7$ | ~ 380 ug |
| Hela | ≒ 1 x $10^7$ | ~ 1300 ug |

| Tissue type | Amount of Starting material | Average yield of Total Protein |
|---|---|---|
| Liver (rat) | ≒ 50 mg | ~ 11.0 mg |
| Kidney (rat) | ≒ 50 mg | ~ 6.5 mg |
| Lung (rat) | ≒ 50 mg | ~ 8.1 mg |
| Heart (rat) | ≒ 50 mg | ~ 9.0 mg |
| Brain (rat) | ≒ 50 mg | ~ 7.6 mg |
| Stomach (rat) | ≒ 50 mg | ~ 10.0 mg |
| Spleen (rat) | ≒ 20 mg | ~ 5.8 mg |