Insect PopCulture® Reagent

Description

Insect PopCulture Reagent	50 ml	71187-3
	250 ml	71187-4

Insect PopCulture Reagent is a buffered mixture of concentrated detergents formulated to extract proteins from insect cells directly in their culture medium. During a 15 minute incubation, Insect PopCulture disrupts the cell membrane without denaturing proteins and protects them from the pH extremes in high-density culture media. Insect PopCulture simplifies sample preparation and is compatible with a number of protein analysis and purification methods, making it an ideal reagent for rapid screening. To reduce viscosity, Benzonase Nuclease® may be added to the reagent. Benzonase degrades endogenous nucleic acids that may interfere with processing due to high viscosity and interaction with proteins of interest.

Insect PopCulture can be used for suspension cultures or adherent cells. The target protein can be extracted from the cells with Insect PopCulture and directly purified from the total culture extract in the original culture flask or plate. Yields are generally higher than those obtained from cell pellets because the total culture also contains proteins released into the medium through secretion or cell lysis (1).

The Insect PopCulture extraction procedure is ideally suited to high throughput (HT) robotic processing of samples for proteomics research or any application that would benefit from the increased yield, speed and convenience. Insect PopCulture extraction and purification in a 96-well format can be achieved with Insect RoboPop™ Ni-NTA His•Bind® Purification Kit (Cat. No. 71257-3).

Storage

Store Insect PopCulture Reagent at room temperature. Slight turbidity may result if Insect PopCulture is stored at 4°C. If this occurs, equilibrate to room temperature (23°C) and mix by inversion prior to use.

Additional Reagents

Benzonase Nuclease, Purity > 90%	10 KU	70746-3
	2.5 KU	70746-4
Benzonase Nuclease HC, Purity > 90%	25 KU	71205-3

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Protocol

Infection/transfection

Baculovirus infection

1. Seed a shake flask with an appropriate volume of serum-free medium (e.g. 50 ml in a 250 ml flask; 250 ml in a 1 liter flask) containing cells in log phase at 5×10^5 cells/ml. Incubate at 28°C with shaking (150 rpm) until cell density reaches 2×10^6 cells per ml (not higher). If using monolayers for expression, seed a T-75 flask with 2×10^7 cells in 10 ml serum-free medium and incubate until almost confluent.
2. Infect the culture with a recombinant baculovirus at an MOI of at least 5 pfu per cell (> 10 if possible) by adding the appropriate volume of a freshly titered stock of virus.
3. Incubate at 28°C (with shaking at 150 rpm for suspension culture) until time of maximum expression (usually 72 hours).

Transient transfection using Insect GeneJuice® Transfection Reagent

The following protocol describes the procedure for introduction of plasmid DNA into Sf9 cells using Insect GeneJuice Transfection Reagent (Cat. No. 71259), in 10 ml suspension cultures. Alternative volumes require adjustment of the amount of Insect GeneJuice Transfection Reagent and cell seeding densities. For further information see Technical Bulletin 359.

1. Seed 1×10^7 Sf9 cells in 8 ml serum-free medium in a 125 ml Erlenmeyer flask.
2. In a sterile tube, dilute 20 µg plasmid DNA with 1 ml of serum-free medium. Also, dilute 100 µl Insect GeneJuice Transfection Reagent with 1 ml serum-free medium.
3. Add the DNA *dropwise* to the Insect GeneJuice Transfection Reagent and mix immediately by gentle vortexing to avoid precipitation.
4. Incubate at room temperature 15 min.
5. Add the transfection mixture to the cells.
6. Incubate the cells with shaking at 150 rpm at 28°C for 48 h or until maximal target protein expression is achieved.

Insect PopCulture cell extract preparation

1. Add 0.05 culture volume of Insect PopCulture Reagent, followed by 0.4 µl (10 U) Benzonase® Nuclease per 1 ml of the original culture volume.

Note: *Benzonase Nuclease can be pre-mixed with Insect PopCulture Reagent prior to treating the cultures. Pre-mixed Insect PopCulture + Benzonase should be used on the same day and stored at 4°C until use.*

2. Mix by inverting gently several times and incubate 15 min at room temperature.
3. His•Tag® fusion proteins can be purified from total culture extracts using Ni-NTA His•Bind® Resin. Other affinity purification methods are likely to be compatible; however, note that His•Bind Resin (IDA agarose) and GST•Bind™ Resin are not suitable for use with Insect PopCulture extracts.

Note: *Direct analysis of the target protein expression by SDS-PAGE with Coomassie blue staining is possible with highly expressed proteins. Use a maximum load volume for the well with 0.25 volume of 4X SDS Sample Buffer (Cat. No. 70607-3).*

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Additional guidelines for Insect PopCulture Reagent optimization and compatibility

Medium: TriEx™ Insect Cell Medium (Cat. No. 71022-3) and BacVector® Insect Cell Medium (Cat. No. 70590-3) have been used successfully with Insect PopCulture. Other serum-free media are likely to be compatible. Serum may interfere with downstream applications such as protein assays and purification.

Cell Lines: TriEx Sf9 Cells and Sf9 Insect Cells have been used successfully with Insect PopCulture. Other insect lines should be compatible, though it is possible that line-dependent differences could occur.

Sf9 Insect Cells (Cat. No. 71104-3) plus BacVector Insect Cell Medium are recommended for transfection and baculovirus plaque assays. TriEx Sf9 Cells (Cat. No. 71023-3) are derived from a high-yielding clone of Sf9 cells and are pre-adapted for growth in TriEx Insect Cell Medium. This matched cell/medium combination has been selected based on rapid, vigorous cell growth and high protein expression levels when using recombinant baculovirus for protein production.

Benzonase® Nuclease: Cell extracts may become viscous from nucleic acids released during cell lysis. These nucleic acids can interfere with effective protein purification. Benzonase degrades all forms of DNA and RNA (single stranded, double stranded, linear and circular) to 5'-monophosphate terminated oligonucleotides 2–5 bases long (2, 3). Although Benzonase requires Mg^{2+} for activation, it does not appear to require additional Mg^{2+} under the conditions described here. The activity is sufficient for effective viscosity reduction and nucleic acid digestion. Benzonase treatment is not generally recommended for purification of proteins that must be nuclease-free. However, depending on the processing methods, Benzonase may be removed during purification by anion exchange chromatography. Residual nuclease activity can be checked by incubation of the purified protein with RNA or DNA markers followed by gel analysis. For further details on Benzonase Nuclease®, see Technical Bulletin 261.

Temperature of extraction: Insect PopCulture extraction along with Benzonase treatment can be performed at room temperature or at 4°C. However, incubation times may need to be increased because Benzonase activities are decreased at lower temperatures.

pH of Extraction: Acidic pH (< 5.0) can degrade components of Insect PopCulture.

Reducing agents: Insect PopCulture is compatible with reducing agents such as 2-mercaptoethanol and DTT. Note that reducing agents may activate proteases and will interfere with protein binding to some purification resins (up to 20 mM 2-mercaptoethaol or up to 1 mM THP [Tris(hydroxypropyl)phosphine] may be used with Ni-NTA His•Bind® Resin).

EDTA: EDTA is compatible with Insect PopCulture. Although not recommended, up to 1 mM EDTA may be used with Ni-NTA His•Bind Resin. Note that, Benzonase Nuclease is inhibited by EDTA at concentrations > 1 mM due to chelation of essential Mg^{2+} ions.

Protein assays: Because proteins generally retain their activity and conformation, protein specific activity and immunoassays are likely to be compatible with Insect PopCulture extraction. The S•Tag™ Rapid Assay, FRETWorks™ S•Tag Assay and BCA protein assays are compatible.

Protease inhibitors: Protease inhibitors may be added with the Insect PopCulture Reagent to the culture. Serine protease inhibitors should be avoided if the target protein is to be treated with the serine proteases Thrombin (Cat. No. 69671-3), Factor Xa (Cat. No. 69036-3) or Recombinant Enterokinase (Cat. No. 69066-3). Active inhibitor may be carried through the purification process and affect cleavage reactions. Note that protease inhibitor cocktails that produce a final concentration of > 1 mM EDTA would not be compatible with Ni-NTA His•Bind Resin or Benzonase treatment.

Purification resins: The Insect PopCulture Reagent contains Tris buffer and is compatible with Ni-NTA His•Bind purification systems at a neutral pH (1) (See Technical Bulletin 273). His•Bind (IDA agarose) and GST•Bind Resins are NOT compatible with purification of proteins from Insect PopCulture extracts. Insect PopCulture is expected to be compatible with many other affinity purification that are not affected by components in the culture medium. Attention should be paid to any observed precipitation of Insect PopCulture or target protein, and compatibility of the Insect PopCulture with buffers used for equilibration of chromatography columns.

References

1. Loomis, K., Grabski, A. and Wong, S. (2002) *in**Nova**tions* **15**, 16–17.
2. Nestle, M. and Roberts, W.K. (1969) *J. Biol. Chem.* **244**, 5213–5218.
3. Janning, P., Schrader, W. and Linscheid, M. (1994) *Rapid Commun. Mass Spectrom.* **8**, 1035–1040.

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