



SF9 Insect Cell Host Cell Proteins

Western Blot Kit for the Detection of SF9 Insect Cell Host Cell Proteins Catalog # F125

Intended Use

This kit is intended for use in determining the presence of host cell protein contamination in products manufactured by recombinant expression in SF9 (*Spodoptera frugiperda*) host cells. The kit is for Research and Manufacturing use only and is not intended for diagnostic use in humans or animals.

Summary and Explanation

Recombinant expression using the vector *Baculovirus* transfected into SF9 insect cells has been shown to be an effective procedure to manufacture cost effective quantities of a desired protein. Many of these recombinantly produced proteins are intended for use as therapeutic agents in humans and animals and as such must be highly purified. The manufacturing and purification process of these products leaves the potential for contamination by host cell proteins from SF9. Such contamination can result in adverse toxic or immunological reactions and thus it is desirable to reduce host cell contamination to the lowest levels practical.

The Western blot technique is a common analytical tool used to detect host cell protein contamination. Samples to be evaluated are first subjected to polyacrylamide gel electrophoresis (PAGE) often in the presence of detergent such as SDS and a reducing agent such as dithiothreitol (DTT). Under these conditions, proteins will migrate through the gel and be separated as a function of their mass and charge. In the Western Blot procedure the proteins which are separated on the gel are then electrophoretically transferred to a membrane, typically made of nitrocellulose or polyvinylidene difluoride (PVDF) where these proteins are essentially irreversibly adsorbed onto the membrane. After a blocking step with an irrelevant protein such as bovine serum albumin to saturate unoccupied adsorption sites on the membrane, the membrane is then exposed to a solution containing a rabbit anti-SF9 antibody conjugated to biotin. This antibody will in turn bind to any transferred proteins for which they are specific. After washing the membrane to remove any unbound antibody the membrane is incubated with a solution containing streptavidin labeled with the enzyme Alkaline Phosphatase followed by another wash step. The membrane is then incubated with a chromogenic, precipitating substrate for Alkaline Phosphatase. Those locations where streptavidin has bound to biotin antibody in turn bound to host cell proteins will be indicated by the generation of a blue substrate chromogen product in characteristic bands on the membrane. In this way, specific components in a complex mixture of proteins can be conclusively identified.

The antibodies used in this kit are polyclonal and were generated by a proprietary procedure designed to elicit a very broad reactivity to a large number of SF9 antigens. These antibodies have been shown to react to more than 40 different SF9 protein bands. This kit provides a simple, very sensitive system capable of detecting as little as 1 ng of protein per band.

As such this kit can be used as a process development tool or routine quality control methods to monitor the optimal removal of host cell contaminants.

For more sensitive detection of SF9 HCP's in downstream or final product it is recommended to use an ELISA. In addition to almost 2 log orders of increased sensitivity the ELISA is also more quantitative. Cygnus Technologies also has available an SF9 HCP ELISA kit, Cat. # F020.

Reagents & Materials Provided

Component	Product #
Anti-SF9 Biotinylated	F126
Affinity purified rabbit antibody, biotinylated in a protein matrix with preservative, 2x50 mL	
SF9 Control Antigen	F127
Solubilized SF9 Insect Cell proteins with preservative, 1x 50µL at ~ 0.5mg/mL total HCP.	
Streptavidin: Alkaline Phosphatase	F009
In a protein matrix with preservative, 2x50mL	
Block/Wash Concentrate	F062
20 fold concentrate of a Tris buffered saline solution with bovine serum albumin and preservative, 1 x 50 mL	
BCIP/NBT Substrate	F064
5-bromo-4-chloro-3-indolyl-phosphate & nitroblue tetrazolium, '1,2 Propanediol', 1x100mL	

Storage & Stability

- * All reagents should be stored a 2°C to 8°C for stability until the expiration date printed. **DO NOT FREEZE.**
- * Reconstituted wash solution is stable until the expiration date of the kit.

Materials Required But Not Provided

- Blotting/Transfer membranes (nitrocellulose or PVDF)
- Distilled water
- 1 liter container for wash solution storage
- Reagent trays

Precautions

For research or manufacturing use only. At the concentrations used in this kit none of the reagents are believed to be harmful. This kit should only be used by qualified technicians.

Preparation of Reagents

- * Bring all reagents to room temperature.
- * Dilute wash concentrate to 1 liter in distilled water, and label with kit lot and expiration date and store at 4°C.

Procedural Notes

Complete washing of the membrane to remove excess unreacted rabbit SF9:biotin and Streptavidin: alkaline phosphatase is essential to minimize background color and achieve maximum sensitivity.

Limitations

1. The antibodies were generated against a laboratory strain of *SF9* cells commonly used in recombinant procedures. A typical solubilized preparation of *SF9* can show more than 40 distinct bands. However, there can be no guarantee that this assay will detect all proteins or protein fragments from *SF9*.
2. Typical Western Blot sensitivity limits for detection of *SF9* proteins are approximately 1ng per band. The detection limits for some bands could be higher than 1ng per band.
3. It is recommended that other methods of host cell protein contamination detection such as ELISA be evaluated to ensure the absence of significant contamination.

Blotting Protocol Guidelines

- * Optimization of the conditions for the PAGE and electrophoretic transfer to the membrane needs to be experimentally determined by each user in order to achieve maximum sensitivity for the Western Blotting procedure.
- * The following procedure is typical of one which might be used to give satisfactory results on 8x10cm mini-gels. This procedure is offered as an example only. You may find it advantageous to vary reagent volumes, incubation times and washing steps to achieve the desired results.
- * The *SF9* Control Antigen is previously solubilized HCPs and is provided to serve as a positive control for the entire procedure from electrophoresis to completion of the blotting protocol. This material has an approximate total HCP concentration of 0.5 mg/mL. As such, the user may find it helpful to dilute this material further to maximize band discrimination. The control antigen should be treated in the same way as samples, i.e. dilution in reducing or non-reducing PAGE running buffers.

Protocol for Minigel (8x10cm) Blots

1. After electrophoretic transfer from the PAGE gels onto the membrane, place the membrane into 40mL of diluted Block/Wash solution in an appropriately sized reagent tray. Allow the blocking of the membrane to proceed for 30 minutes with agitation or rotation to ensure good mixing and even diffusion through the membrane.
2. Pour off the Block/Wash solution and add 20mL* of rabbit biotinylated anti-SF9 (#F126). Incubate with gentle agitation for 1 hour at room temperature.
3. Carefully pick up the membrane by the corner using forceps. Touch-off any drops of the antibody solution and transfer to a clean reagent tray containing 40mL of Block/Wash solution. Allow the membrane to wash for 5 minutes with agitation. Pour off the Block/Wash solution and replace with another 40mL. Repeat for a total of 4 washes.
4. Transfer the membrane to a clean tray, add 20 ml of Streptavidin:Alkaline Phosphatase (#F009) and incubate with gentle agitation for approximately 30 minutes.
5. Repeat the 4 wash steps as described in Step 3.
6. Transfer the membrane to a clean reagent tray containing 20mL of the BCIP/NBT substrate. Incubate with gentle agitation for approximately 1 hour.**
7. Stop the substrate by rinsing the membrane in distilled water.

*The reagent tray should be a length and width such that the volume of enzyme conjugate added will completely cover the membrane and allow for free flowing of the solution around the membrane.

The point at which to stop the substrate incubation should be determined by the user for each blot. The reaction should be stopped before the background color becomes so intense that there is insufficient contrast between positive bands and background.

Ordering Information & Customer Service

To place an order or to obtain additional product information contact *Cygnus Technologies* Customer Support:

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