Chinese Hamster Ovary Host Cell Proteins

Western Blot Kit for the Detection of
Chinese Hamster Ovary Host Cell Proteins
Catalog # F060

Intended Use

This kit is intended for use in determining the presence of Chinese Hamster Ovary (CHO) protein contamination in products manufactured by recombinant expression in CHO 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 by CHO is a widely used procedure to obtain sufficient and 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 CHO. 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 dithiotreitol (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 antibodies. These antibodies will in turn bind to any transferred proteins for which they are specific. If the antibodies are labeled directly or indirectly with a reporter molecule such as an enzyme, a substrate can then be reacted with the membrane. Those locations where the enzyme labeled antibody has bound to a transferred protein will be indicated by the generation of a 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 CHO antigens. These antibodies have been shown to react to more than 40 different CHO protein bands from SDS/DTT solubilized CHO cells. The antibodies have been further affinity purified and then directly conjugated with the enzyme Alkaline Phosphatase. This provides a simple, direct and 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.

Reagents & Materials Provided

Component	Product #
Anti-CHO:Alkaline Phosphatase Conjugate Affinity purified goat antibody conjugated to alkaline phosphatase in a protein matrix with preservative. 2x50mL	F061
CHO Control Antigen Mixture of more than 20 CHO proteins solubilized in SDS/DTT with preservative. 1x50µL	F063
BCIP/NBT Substrate 5-bromo-4-chloro-3-indolyl-phosphate & nitroblue tetrazolium. 1x100mL	F064
Block/Wash Concentrate (20X) Tris buffered saline with bovine serum albumin and preservative. 1x50 mL	F062

Storage & Stability

* All reagents should be stored at 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, label with kit lot and expiration date, and store at 4°C.

Procedural Note

Complete washing of the membrane to remove excess unreacted goat CHO:Alkaline Phosphatase is essential to minimize background color and achieve maximum sensitivity.

Limitations

1. The antibodies were generated against a blend of two CHO cell lines commonly used in recombinant procedures. A typical SDS/DTT solubilized preparation of CHO cells can show more than 40 distinct bands. However, there can be no guarantee that this assay will detect all proteins or protein fragments from CHO.

2. Typical Western Blot sensitivity limits for detection of CHO 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 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 need 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 CHO Control Antigen is provided to serve as a positive control for the entire procedure from electrophoresis to completion of the blotting protocol. It is recommended to apply 5µL of this material to one of the gel lanes. The development of at least 20 bands during the substrate step is indicative of a satisfactory run.

Typical 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 CHO:Alkaline Phosphatase (#F061). 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:enzyme conjugate 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 reagent tray containing 20mL of the BCIP/NBT substrate (#F064). Incubate with gentle agitation for approximately 1 hour.**

5. Stop the substrate by rinsing the membrane in distilled water.

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* 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*:

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