E. coli Host Cell Proteins

Western Blot Kit for the Detection of *E. coli* Host Cell Proteins Catalog # F415

Intended Use

This kit is intended for use in determining the presence of *E.coli* Host Cell Protein contamination in products manufactured by recombinant expression in *E.coli* 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 in *E.coli* is a relatively simple and cost effective method for production of therapeutic proteins and pDNA. Many of these recombinantly produced products 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 (HCP) from *E.coli*. 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 characterize complex protein solutions and anti-HCP antibodies. Western blot will often be able to detect HCP in samples upstream in the purification process 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 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 goat antibodies to *E.coli* labeled with the enzyme Horse Radish Peroxidase (HRP). These antibodies will in turn bind to any transferred proteins for which they are specific. After a wash step to remove any non-immunologically bound antibody, the membrane is finally exposed to the substrate, Tetramethylbenzidine (TMB) specifically formulated to precipitate on 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 *E. coli* antigens. These antibodies have been shown to react to more than 40 different *E. coli* protein bands from SDS/DTT solubilized *E. coli* cells, or from HCP's found in conditioned *E. coli* culture media after one dimensional PAGE separation. This kit provides a simple, very sensitive system capable of detecting as little as 1ng of protein per band. As such this kit can be used as a process development tool or routine quality control method to monitor the optimal removal of host cell contaminants. Because of sensitivity limitations in the method, Western blot is normally not sensitive enough to detect HCP contamination in downstream or final product. For more sensitive detection of *E. coli* HCP's in downstream or final product it is recommended to use an ELISA. In addition, to more than 2 log orders of improved sensitivity over typical Western Blots, an ELISA is more quantitative and subject to less product interference. *Cygnus Technologies* also sells an *E. coli* HCP ELISA kit, Cat. No. F410, that uses the same antibodies as are employed in this Western Blot kit.

Reagents & Materials Provided

Component	Product #
Anti-*E. coli*:HRP Conjugate Affinity purified goat antibody conjugated to HRP in a protein matrix with preservative. 2x50mL	**F416**
***E. coli* Control Antigen** Solubilized and diluted *E. coli* cell proteins with preservative. 1 x 50µL	**F417**
TMB Substrate 3,3',5,5' Tetramethylbenzidine. 1x100mL	**F129**
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

* Prior to PAGE, the Control Antigen (#F417), can be diluted in the same buffer (reducing or non-reducing) as the samples.

* 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 *E. coli*:HRP is essential to minimize background color and achieve maximum sensitivity.

Limitations

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

2. Typical Western Blot sensitivity limits for detection of *E. coli* 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. *Cygnus Technologies* also manufactures a microtiter plate based ELISA for *E. coli*, Cat. No. F410. The ELISA is approximately 100 fold more sensitive than the Western Blot and as such is more suitable for final product testing where HCP levels will typically be below the sensitivity limits of Western Blot.

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 that 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, antibody:enzyme conjugate dilution, incubation times and washing steps to achieve the desired results.

* The *E. coli* Control Antigen is a diluted extract provided to serve as a positive control for the entire procedure from electrophoresis to completion of the blotting protocol. This material should be treated in the same way as samples, i.e. dilution in reducing or non-reducing PAGE running buffers. Recommended final dilution of the control antigen is 1:1 to 1:5. 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 Anti-*E. coli*:HRP Conjugate (#F416). Incubate with gentle agitation for 2 hours 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 TMB substrate (#F129). Incubate with gentle agitation for approximately 30 minutes.**

5. 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. In some cases sensitivity can be increased by incubating with the anti-*E. coli*:HRP for up to 16 hours. If it is necessary to stop the substrate reaction much earlier than 30 minutes the user may consider diluting the antibody:enzyme conjugate in Block/Wash Solution or shortening the immunological incubation step to less than 2 hours.**

Ordering Information/ Customer Service

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

www.cygnustechnologies.com
Cygnus Technologies, Inc.
4701 Southport Supply Rd. SE, Suite 7
Southport, NC 28461 USA
Tel: 910-454-9442
Fax: 910-454-9443
Email: techsupport@cygnustechnologies.com
