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Saccharomyces cerevisiae Host Cell Proteins

Western Blot Kit for the Detection of Saccharomyces cerevisiae Host Cell Proteins Catalog # F130

Intended Use

This kit is intended for use in determining the presence of *Saccharomyces cerevisiae* protein impurities in products manufactured by recombinant expression in this cell line. 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 *Saccharomyces cerevisiae* is an efficient method to obtain 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 impurities by host cell proteins from *Saccharomyces cerevisiae.* Such impurities can result in adverse toxic or immunological reactions and thus it is desirable to reduce host cell impurities to the lowest levels practical.

The Western blot technique is a common analytical tool used to detect host cell protein impurities. 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 a goat anti- *Saccharomyces cerevisiae* 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 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 antibody used in this kit is polyclonal and was generated by a proprietary procedure designed to elicit a very broad reactivity to a large number of *Saccharomyces cerevisiae* antigens. These antibodies have been shown to react to more than 40 different *Saccharomyces cerevisiae* protein bands from SDS/DTT solubilized *Saccharomyces cerevisiae* cells after one dimensional PAGE separation. This assay provides a simple and 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 impurities.

Reagents & Materials Provided

Component	Product #
Anti-*S.cerevisiae*, biotinylated Affinity purified goat antibody biotinylated in a protein matrix with preservative. 2x50mL	F134
***S.cerevisiae* Control Antigen** *S.cerevisiae* cells solubilized and diluted with preservative. 1x50µL	F132
Streptavidin:Alkaline Phosphatase In a protein matrix with preservative. 2x50mL	F009C
BCIP/NBT Substrate 5-bromo-4-chloro-3-indolylphosphate & nitroblue tetrazolium. 1x100mL	F064
Block/Wash Concentrate (20X) Tris buffered saline with bovine serum albumin and preservative. 2x50mL	F062

Storage & Stability

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

Materials & Equipment Required But Not Provided

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

Precautions

- For Research or Manufacturing use only.
- This kit should only be used by qualified technicians.

Preparation of Reagents

- Prior to PAGE, the Control Antigen (#F132), should be diluted in the same running buffer (reducing or non-reducing) as the samples.
- Bring all reagents to room temperature.
- Dilute wash concentrate to 2 liters 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 anti-*S.cerevisiae* antibody and streptavidin:alkaline phosphatase is essential to minimize background color and achieve maximum sensitivity.

Limitations

1. The goat antibody was generated against a solubilized preparation from whole *Saccharomyces cerevisiae* cells treated to enhance the immunogenicity of the various antigens and to etensure broad reactivity to multiple *Saccharomyces cerevisiae* proteins. However there can be no guarantee that this assay will detect all proteins or protein fragments from *Saccharomyces cerevisiae*. A typical SDS/DTT solubilized preparation of *Saccharomyces cerevisiae* cells can show more than 40 distinct bands.

2. Typical Western Blot sensitivity limits for detection of *S.cerevisiae* 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 pollutants be evaluated to ensure the absence of significant impurities.

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 PAGE gel formulations, reagent volumes, incubation times and washing steps to achieve the desired results.
- The *S.cerevisiae* Control Antigen is 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 1:3. The development of at least 15 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 biotinylated anti-*S.cerevisiae* (#F134). 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 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 Streptavidin:Alkaline Phosphatse (#F009C). 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 BCIP/NBT substrate (#F064). 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*:

www.cygnustechnologies.com
Cygnus Technologies, LLC
4332 Southport Supply Rd. SE
Southport, NC 28461 USA
Tel: 910-454-9442
Fax: 910-454-9443
Email: techsupport@cygnustechnologies.com