

Pichia pastoris Host Cell Proteins

Western Blot Kit for the Detection of *Pichia pastoris* Host Cell Proteins Catalog # F145

Intended Use

This kit is intended for use in determining the presence of *Pichia pastoris* Host Cell Protein impurities in products manufactured by recombinant expression in *Pichia pastoris* 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 *Pichia pastoris* cell line 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 pollutants by host cell proteins from *Pichia pastoris*. Such pollutants 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 pollution. 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 goat antibodies to *Pichia*

pastoris which have been 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 a substrate specific to HRP, called Tetramethylbenzidine (TMB). 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 *Pichia pastoris* antigens. These antibodies have been shown to react to more than 30 different *Pichia pastoris* protein bands from SDS/DTT solubilized *Pichia pastoris* cells. 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 methods to monitor the optimal removal of host cell pollutants.

Reagents & Materials Provided

Component	Product #
Anti-<i>Pichia pastoris</i>:HRP Conjugate Affinity purified goat antibody conjugated to HRP in a protein matrix with preservative. 2x50mL	F147
<i>Pichia pastoris</i> Control Antigen Solubilized <i>Pichia pastoris</i> cell proteins with preservative. 1x50µL	F146
TMB Substrate 3,3',5,5' Tetramethylbenzidine. 1x100mL	F129
Block/Wash Concentrate (20X) Tris buffered saline with bovine serum albumin and preservative. 1x50mL	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
- 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 *Pichia pastoris*:HRP is essential to minimize background color and achieve maximum sensitivity.

Limitations

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

2. Typical Western Blot sensitivity limits for detection of *Pichia pastoris* 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 impurities be evaluated to ensure the absence of significant pollution. *Cygnus Technologies* also manufactures a microtiter plate based ELISA for *Pichia pastoris*, Cat. No. F140. This kit 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 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 *Pichia pastoris* 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. It is not intended to demonstrate the full immunoreactivity of the antisera. The user should evaluate a concentrated source of their *Pichia* proteins to determine reactivity to their strain. The control antigen should be treated in the same way as samples, i.e. dilution in 2x reducing or non-reducing PAGE sample 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-*Pichia pastoris*:HRP Conjugate (#F147). 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 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 1 hour.**
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- *Pichia pastoris*:HRP for up to 16 hours.

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