

## Goat Milk Proteins

# Western Blot Kit for the Detection of Goat Milk Proteins Catalog # F245

### Intended Use

This kit is intended for use in determining the presence of goat milk protein contamination in products manufactured by transgenic expression in goat milk. The kit is for Research and Manufacturing use only and is not intended for diagnostic use in humans or animals.

### Summary and Explanation

Transgenic expression of therapeutic proteins in milk is a cost effective method for production of large quantities of therapeutic proteins. As therapeutic agents these proteins must be highly purified. The manufacturing and purification process of these products leaves the potential for contamination by other proteins normally found in milk. Such contamination can reduce the efficacy of the therapeutic agent and result in adverse toxic or immunological reactions and thus it is desirable to reduce the milk protein 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 to saturate unoccupied adsorption sites on the membrane, the membrane is then exposed to a solution containing rabbit antibodies to goat milk proteins 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 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 milk proteins. These antibodies have been shown to react with more than 30 different bands 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 milk protein contaminants. For more sensitive detection of milk proteins in downstream or final product it is recommended to use an ELISA. *Cygnus Technologies* also has available a Goat Milk ELISA kit, # F240, for semi-quantitative detection.

While Western Blot is a powerful method it suffers from a number of limitations. Western Blot is a complex and technique dependent procedure requiring a subjective interpretation of results. Furthermore it is essentially a qualitative method which does not lend itself to obtaining quantitative answers. The sensitivity of Western Blot is severely limited by the volume of sample which can be tested and by interference from the presence of high concentrations of the intended product. As such Western Blot is normally only adequate to detect milk protein contamination in upstream purification process samples.

### Reagents & Materials Provided

<u>Component</u>	<u>Product #</u>
<b>Anti-Goat Milk:HRP Conjugate</b> Affinity purified rabbit antibody conjugated to HRP in a protein matrix with preservative, 2x50 mL	<b>F246</b>
<b>Goat Milk Control Antigen</b> Solubilized and diluted goat milk proteins with preservative, 1 x 50µL	<b>F247</b>
<b>Block/Wash Concentrate</b> 20 fold concentrate of a Tris buffered saline solution with blocking protein and preservative, 1 x 50 mL	<b>F062</b>
<b>TMB</b> 3,3',5,5' Tetramethylbenzidine, 1 x 100mL	<b>F129</b>

## 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

- \* Prior to PAGE, the Control Antigen, #F247 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 1 liter in distilled water, and label with kit lot and expiration date and store at 4°C.

## Procedural Note

Complete washing of the membrane to remove excess unreacted anti-goat milk:HRP conjugate is essential to minimize background color and achieve maximum sensitivity.

## Limitations

1. The antibodies were generated against clarified goat milk. A typical SDS/DTT PAGE separation of goat milk can show more than 30 distinct bands. However, there can be no guarantee that this assay will detect all proteins that might be present in milk.
2. Typical Western Blot sensitivity limits for detection of milk 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 analytical methods be evaluated to ensure the absence of significant milk protein contamination. *Cygnus Technologies* also manufactures a microtiter plate based ELISA for goat

milk proteins, Cat. No. F240. This kit is approximately 100 fold more sensitive than the Western Blot and as such is more suitable for final product testing where milk protein levels will typically be below the sensitivity limits of Western Blot.

## 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-Goat Milk:HRP Conjugate (#F246). 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 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.

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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. In some cases sensitivity can be increased by incubating with the anti-goat milk: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.

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

\* The Goat Milk Control Antigen is a diluted extract which 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. Depending upon assay conditions used, optimal resolution of milk protein bands may be enhanced by further dilution of the control antigen. It is recommended that initial blots test the control antigen neat and down to dilutions of approximately 1:10 to establish optimal concentration.

### **Ordering Information & Customer Service**

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

**Tel: 508-910-454-9442    FAX: 910-454-9443  
Email: [cygnustec@aol.com](mailto:cygnustec@aol.com)  
Web site: [www.cygnustechnologies.com](http://www.cygnustechnologies.com)  
4701 Southport Supply Road SE, Suite 7  
Southport, NC 28461 USA**