Antibody Stripping Buffer

From Gene Bio-Application Ltd.

Cat. No.	Description
ST010	**Immunodetection removing buffer, 500 ml**
ST013	**Immunodetection removing buffer, 30 ml**

Storage and Shipping Instruction: Shipped at ambient temperature. Stable at room temperature (22-25°C) for 1 year.

This product is guaranteed for one year from date of purchase when properly handled and stored.

Introduction

Western Blot is widely used to detect and compare proteins from a complex mixture utilizing antibody detection on a membrane. Chemiluminescence has become an easy and sensitive method of detection compared to other analysis. Because of the nature of chemiluminescence detection, it is possible to **reprobe** the separated protein mixture on the membrane. Conventionally, Western Blots have been stripped using extremely harsh conditions that may alter the antigen for subsequent immunoprobing. Gene Bio-Application, Stripping Buffer is a novel formula provides a gentle method of removing primary and secondary antibodies from membranes that allows several reprobings on the same membrane.

Protocol

IMPORTANT: Optimization of incubation time is essential for best results.

IMPORTANT: If the blot cannot be stripped immediately after chemiluminescence detection, the blot can be stored in PBS or TBST at 4°C until the stripping procedure is to be preformed.

1. Place the blot to be stripped in the Stripping Buffer. Add Stripping Buffer, to fully immerse the blot**.**

2. Incubate the blot in the Striping Buffer for 5-15 min at room temperature with vigorous shaking**.**

IMPORTANT: In general, higher affinity antibodies or large quantities of detected protein will require longer incubation time for stripping.

3. Empty the Stripping Buffer.

4. To wash, add 300 ml of dH2O and shake vigorously for 5 min**.**

5. Repeat Steps 4 five more times.

Monitoring of complete removal of label secondary antibody: After Step 5 incubate the membrane with fresh chemiluminescence reagents and expose to film. If no signal is detected with a 5 min exposure, the label secondary conjugate has been successfully removed from the antigen or primary antibody.

Monitoring of complete removal of primary label antibody :
After Step 5 incubate the membrane with the label secondary antibody, followed by a wash in wash buffer. Incubate the membrane with fresh chemiluminescence reagents and expose to film. If no signal is detected with a 5 min exposure, the primary antibody has been successfully removed from the antigen.

IMPORTANT: Analysis of the successful removing of immunoprobes is recommended to prevent removal of the antigen or the unsuccessful removal of the antibodies.

6. If signal is detected in the two experiments describe above, place the blot back into Stripping Buffer for additional 5-15 min.
7. After it has been determined that the membrane is free of the immunodetection reagents, a second immunoprobing can take place.

Start the second immunoprobing with reblocking of the blot.

IMPORTANT: The blot can be stripped up to 5 times. However, longer exposure times or more sensitive chemiluminescence substrate is needed. Actually, re-probing may result in a decrease in signal if antigen is unstable. Analysis of the individual system is required.

More Information is available in our Web. Site www.geba.org.