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. Upon arrival, store at 4°C. Stable at room temperature or 4°C for 1 year.

IMPORTANT: Allow the buffer to warm to room temperature before use.

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 methods of 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. The Gene Bio-Application Stripping Buffer is a novel formula providing a gentle method of removing primary and secondary antibodies from membranes that allows reprobing the same membrane several times.

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 performed.

1. Place the blot to be stripped in the Stripping Buffer. Top up Stripping Buffer until the blot is completely immersed.

2. Incubate the blot in the Striping buffer for 5-15 min at room temperature under vigorous shaking.

   IMPORTANT: In general, higher affinity antibodies or large quantities of detected protein will require longer incubation time for stripping. **However, each half-minute is critical to achieve optimum incubation time.**

3. Empty the Stripping Buffer.
4. To wash, add 300 ml of dH_2O and shake vigorously for 5 min.
5. Repeat Step 4 five more times.

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

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

   IMPORTANT: Analysis of successful removal 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 described above, place the blot again in Stripping Buffer for additional 5-15 min.
7. After it has been determined that the membrane is free of the immunodetection reagents, a second immunoprobe can take place. **Start the second immunoprobe 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 the antigen is unstable. Analysis of the individual system is required.