SureSignal Western Blotting ECL Substrates

Introduction

The SureSignal range of Western blotting substrates are luminol-based enhanced chemiluminescent substrates which are compatible with immunoblotting methods employing horseradish peroxidase (HRP) – conjugated secondary antibodies.

Detection of picogram to low femtogram amounts of antigen is possible due to the excellent sensitivity and long signal duration of the SureSignal Western Substrates. The long duration of the chemiluminescent signal enables both digital and film-based imaging, without any loss of signal.

Contents

This manual covers the following SureSignal reagents:

Catalog number	SureSignal substrate	Size
LU321-B100ML	SureSignal Pico Plus Western Substrate	50 ml Luminol Solution 50 ml Peroxide Solution
LU345-B100ML	SureSignal Pico Ultra Western Substrate	50 ml Luminol Solution 50 ml Peroxide Solution
LU365-B100ML	SureSignal Femto Western Substrate	50 ml Luminol Solution 50 ml Peroxide Solution

Storage

Store at 4°C. Stable for 2 years upon receipt.

Safety

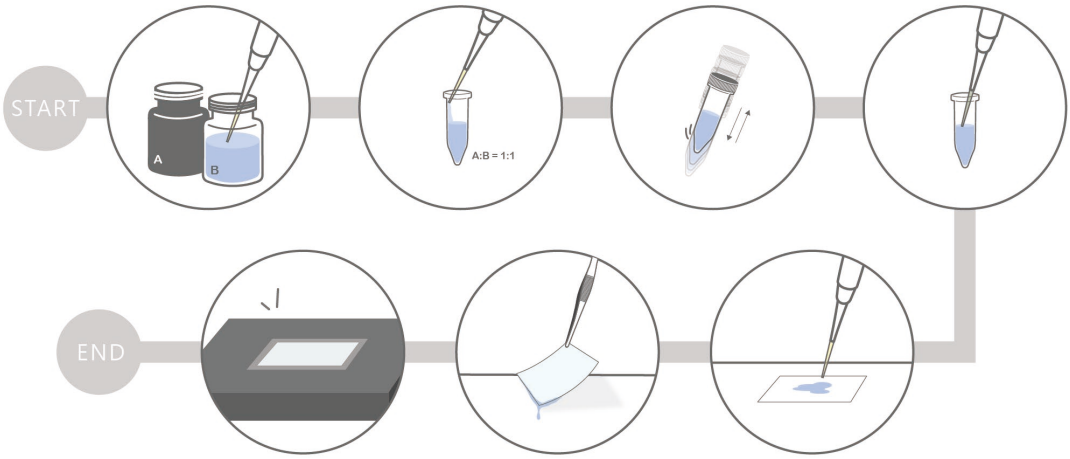
Please consult the MSDS available on our website for safety instructions or contact us at info@lubio.ch or +41 41 417 02 80. Always wear appropriate protective clothing, eye protection glasses and gloves when handling SureSignal substrates.

Recommendations for use

SureSignal substrate	Sensitivity range (antigen)
SureSignal Pico Plus Western Substrate	Low picogram to high femtogram
SureSignal Pico Ultra Western Substrate	Low picogram to mid femtogram
SureSignal Femto Western Substrate	Mid femtogram to low femtogram

Protocol

1. Keep the membrane moist in the wash buffer while preparing the substrate mixture and ensure that the membrane does not dry out during the subsequent steps.
2. Prepare 100 ul of chemiluminescent substrate mix per cm^2 of membrane: mix the Luminol Solution and Peroxide Solution in a 1:1 ratio in an Eppendorf tube and shke thoroughly.
3. Place the membrane with the protein side up in a clean container.
   Remove the membrane from the tray and drain off any excessive chemiluminescent substrate mix.
4. Place the membrane in a plastic sheet protector or in plastic wrap to prevent the membrane from drying out.
   Image the membrane with a digital imager or by exposure to X-ray film.



Troubleshooting

Problem	Cause	Solution
High Background	Overconcentrated primary or secondary antibody	Decrease the antibody concentration
		Perform a dot blot with antigen to optimize the antibody concentration
	Insufficient washing	Increase the frequency or duration of washes
	Incomplete blocking	Increase the concentration of blocking agent in your incubation and washing solutions
		Use SureBlock Western blocking reagent (SB232010)
No Reaction or Weak Signal	Insufficient antigen binding	Decrease antibody concentration Reduce the concentration of blocking agent in your incubation and washing solutions Use SureBlock Western blocking reagent (SB232010)
	Poor antibody binding to antigen	Optimize the detergent used for antibody incubation Increase the antibody incubation time
	Proteins washed from the membrane during assay	Reduce the number or intensity of wash
	Insufficient reagent volume	Apply additional volume of antibody blocking reagent or wash solution

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