GenDEPOT

West-Ez Stripping Buffer

S2100

Storage

Store at room temperature or 4°C

Contents

- Product Manual
- West-Ez Stripping Buffer

ALL PRODUCTS SOLD BY GenDEPOT ARE INTENDED FOR RESEARCH USE ONLY UNLESS OTHERWISE INDICATED. THIS PRODUCT IS NOT INTENDED FOR DIAGNOSTIC OR DRUG PURPOSE

Shipping Condition

Ship at ambient.

Introduction

Nitrocellulose and PVDF membranes that have been probed by Western blotting procedures and detected by chemiluminescent or other nonprecipitating substrates may be stripped and reprobed using West-Ez Stripping Buffer. Traditional stripping methods use conditions that are effective for only low-affinity antibody-antigen interactions or are so harsh conditions that they tend to adversely alter the antigen for subsequent immunoprobing. West-Ez Stripping Buffer provides a generally robust but gentle method for stripping primary and secondary antibodies from blots to enable several reprobings on the same membrane.

Procedure

Note: Blots cannot be stripped immediately after chemiluminescent detection. It should be stored in phosphate buffered saline (PBS) at 4 °C until the stripping procedure can be performed.

1. Warm bottle of West-Ez Stripping Buffer to room temperature.
2. Place the blot to be stripped in West-Ez Stripping Buffer and incubate for 5-15 minutes at room temperature. Use a sufficient volume to ensure that the blot is completely wetted. (i.e., approximately 20ml required for an 8x10 cm blot).

Note: Optimization of both incubation time and temperature is essential for best results. In general, higher affinity antibodies will require at least 15 minutes of stripping and may require an incubation temperature of 37 °C.

3. Remove the blot from the West-Ez Stripping Buffer and wash in Wash Buffer.
4. Test for the removal of the immunodetection reagents.

- Test for complete removal of the HRP label (e.g., secondary antibody):
Incubate the membrane with West-Q Cheminuminescent Substrate Working Solution and expose to film. If no signal is detected using a 5-minute exposure, the HRP conjugate has been successfully removed from the antigen or primary antibody.
- Test for complete removal of the primary antibody:
Incubate the membrane with the HRP-labeled secondary antibody, followed by a wash in wash buffer. Incubate in West-Q Chemiluminescent Working Solution and expose to film. If no signal is detected with a 5-minute exposure, the primary antibody had been successfully removed from the antigen.

5. If signal is detected with either test in step 4, return to step 2, stripping for an additional 5-15 minutes. Some antigen/antibody systems require increased tem -perature and/or longer incubation times to strip then fully. Optimize stripping time and temperature to ensure complete removal of antibodies while preventing damage to the antigen.
6. After determining that the membrane is properly stipped, the second immuno -probing experiment may be performed.

Notes:

- Blot may stipped and reproed several times but may require longer exposure times or a more sensitive chemiluminescent substrate. Subsequent reprobing may result in decresed signal if the antigen is labile in West-Ez Stripping Buffer. Analysis of the individual system is required.
- Reblocking a membrane is usually not necessary after stripping but may be required in some applications.

Related Product

Product Name	Cat No
Agarose Sepro	A0224
100bp PCR Ranger DNA Marker, 100bp-To-30kb	D1108
1kb PCR Ranger DNA Marker, 75bp-to-20kb	D1109
iVDye 50bp DNA Ladder	V1001
iVDye 100bp DNA Ladder	V1002
iVDye 1kb DNA Ladder	V1003
dNTP Mixtures, 10mM Each	D0610
amfiSure PCR Master Mix (2X)	P0311
amfiSure Primer PCR Master Mix (2X)	P1311
amfiSure PCR Master Mix (2X)	P2311
amfiSure Direct PCR Lysis Buffer	D0300
amfiEco Taq DNA Polymerase	P0701
SafePinky DNA Gel Staining Solution	S1001
amfiSure qGreen Q-PCR Master Mix (2X), w/o ROX	Q5600
amfiSure qGreen Q-PCR Master Mix (2X), w Fluorescein	Q5601
amfiSure qGreen Q-PCR Master Mix (2X), High ROX	Q5602
amfiSure qGreen Q-PCR Master Mix (2X), Low ROX	Q5603

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