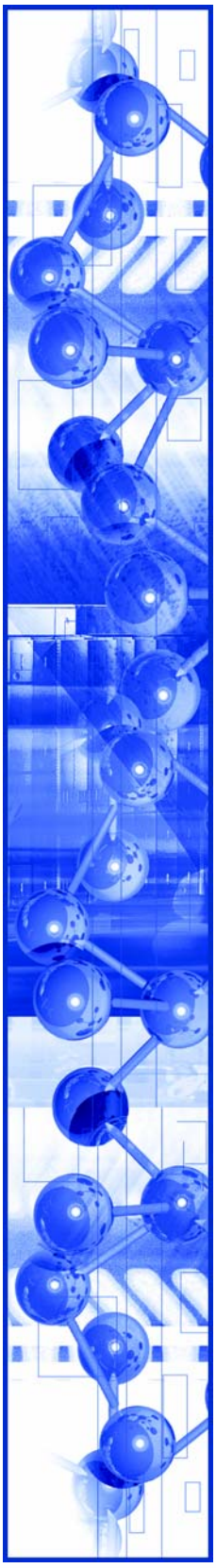

Gentle ReView™ Buffer

A ready-to-use, odor-free buffer for
stripping and reprobing of Western blots

Code	Description
N552-1L	Gentle Review™ Buffer, 1 liter

Description:

Gentle ReView™ Buffer is a mild yet effective method for stripping primary and secondary antibodies from PVDF or nitrocellulose membranes. The gentle nature of this buffer makes it possible to reprobe the same membrane several times without damaging the membrane-bound antigen. It is an odorless, ready-to-use formula that requires no mixing or heating prior to use.

Most antibody:antigen complexes can be dissociated within 30 minutes at room temperature by Gentle ReView™ Buffer. However, incubation times and temperatures are dependent upon the affinity of the antibody:antigen interaction and may need to be optimized for each particular situation.

Background

Western Blotting is widely used to identify specific proteins within a complex mixture by antibody recognition of specific antigenic determinants within the protein. In this procedure, complex samples are resolved into individual protein bands on SDS-polyacrylamide gels and then transferred to a solid support membrane of PVDF or nitrocellulose. The blots are probed with specific antibodies and the bound antibodies are visualized by detection of chemiluminescent or fluorescent substrates.

Detection of multiple antigens within the same sample traditionally requires the generation of multiple blots from multiple gels. This process consumes valuable sample and introduces potential artifacts when comparing blots from different electrophoresis runs. These problems can be eliminated by probing a single blot with multiple antibodies. Unfortunately reagents conventionally used to dissociate antigen-antibody complexes require the use of high temperatures and noxious sulfhydryl reducing reagents to remove bound antibodies. The process often destroys the antigens of interest and damages the membranes leaving them unsuitable for subsequent probing. In contrast, Gentle ReView™ Buffer eliminates the use of harsh stripping reagents and provides an effective, odor-free, stripping buffer that does not damage the blot or the bound antigen.

➥ **Note**: As with other stripping buffers, Gentle ReView™ Buffer for Western Blots will not dissociate the biotin:avidin interaction.

Storage/Stability:

This solution is stable for at least one (1) year at 4°C.

Application Disclaimer

For Research Use Only. Not for Therapeutic or Diagnostic Use.

Protocol for Stripping a Western Blot:

Additional required materials not supplied:

- Western Blot that has been blocked, probed, and detected with a chemiluminescent substrate
- Wash Buffer such as TBS (Tris Buffered Saline, Code J640) or PBS (Phosphate Buffered Saline, Code E404) supplemented with 0.05% Tween® 20 (Code 0777)
- Primary and secondary antibodies for both the first and subsequent Western blotting procedures
- Method for the detection of the chemiluminescent signal

Required Equipment: Shaker

➥**Note**: *Gentle ReView™ Buffer will not remove precipitating detection substrates.*

➥**Note**: *If circumstances do not allow for the immediate stripping of the blot, it can be stored at 4°C in 1X PBS.*

1. Prior to use, equilibrate the Gentle ReView™ Buffer solution to room temperature.
2. After ECL detection, gently rinse the blot in Wash Buffer.
3. Submerge the blot in a sufficient quantity of Gentle ReView™ Buffer solution and completely wet the blot. At least 10 ml of Gentle ReView™ Wash Buffer is recommended for a mini-gel membrane, or about 2.0-2.5 ml/cm^2 of membrane.
4. Incubate the blot in Gentle ReView™ Buffer solution, with rocking or gentle shaking, for approximately 30 minutes at room temperature.

➥ **Note**: *The time and temperature of the incubation depends on the affinity of the antibody:antigen interaction. Strong interactions may need to be incubated at 37°C, or for a longer incubation time at room temperature. Large quantities of detected protein will require longer stripping times. For best results, incubation time and temperature should be optimized or empirically determined for each antibody.*

5. Remove the blot from Gentle ReView™ Buffer solution, and wash several times in Wash Buffer.
6. Testing for Antibody Removal:
   Following the stripping procedure, it is advisable to check for the complete removal of the immunodetection reagents. It is of particular importance if the size of the second antigen to be detected is similar to that of the first.
   a. Test for removal of secondary antibody: Incubate the blot with a freshly prepared working solution of chemiluminescent reagent, such as VisiGlo™ HRP Plus Chemiluminescent Substrate Kit (Code N219) or VisiGlo™ AP Chemiluminescent Substrate (Code N216).
   b. Expose the blot to film for 5 minutes or CCD camera. No signal should be detected.
      - If a signal is present repeat step 3 for a longer incubation time. Incubation can be performed at higher temperatures 30-37º C.
      - If no signal is detectable continue with step c.
   c. Test for removal of the primary antibody: Incubate the blot with the enzyme-conjugated secondary antibody, wash blot and incubate in a freshly prepared working solution of chemiluminescent reagent according to usual procedure.
   d. Expose the blot to film for 5 minutes or CCD camera. No signal should be detected.
      - If a signal is detected return to step 3 and strip the blot for an additional 5-10 minutes. Retest for signal before reprobing.
      - If no signal is detected the blot has been successfully stripped and is ready for another round of immunoprobing.

➥**Note**: ***The blot must be reblocked before beginning new round of probing.***

Additional Notes:

The blot may be stripped and reprobed several times. Subsequent probings may have reduced signal if the antigen is labile or stripping has damaged the antigen on the blot.

References:

1. Kaufmann, Ewing and Shaper (1987). The Erasable Western Blot. *Anal. Biochem.* 161: 89 – 95.

Related Products :

Code	Product
Chemiluminescent Substrates	
N218-KIT	VisiGlo™ HRP Chemiluminescent Substrate
N219-KIT	VisiGlo™ HRP Plus Chemiluminescent Substrate
N216-100ML	Visiglo™ AP Chemiluminescent Substrate
Buffers and Reagents	
E404-200TABS	PBS Tablets, 100 ML
0780-2PK	PBS Powder
J640-4L	TBS Buffer, 20X Liquid
K859-100TABS	TBS Tablets
M235-125G	TBS with Tween 20, Powder, Blend
M228-10ML-5PK	Tween® 20 10% Solution
E671-1L	BIO-BLOCK ™ in 1X PBS
E667-1L	BIO-BLOCK ™ in 1X TBS
M230-42G-5PK	TBS with Non-Fat Powdered Milk
M231-22G-5PK	TBS with BSA
M235-12.5G-5PK	TBS with Tween® 20
M232-39.8G-5PK	PBS with Non-Fat Powdered Milk
M233-19.8G-5PK	PBS with BSA
M245-10.4G-5PK	PBS with Tween® 20
Blotting membranes	
	TotalBLOT+™ Nylon Membranes
E576-5X15CMSQ	15 x 15 cm
E576-10X10CMSQ	10 x 10 cm
E576-1ROLL	30 cm x 3 m (1 Roll)
	TotalBLOT+™ PVDF Membranes
E578-5X15CMSQ	15 x 15 cm
E578-10X10CMSQ	10 x 10 cm
E578-1ROLL	30 cm x 3 m (1 Roll)

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