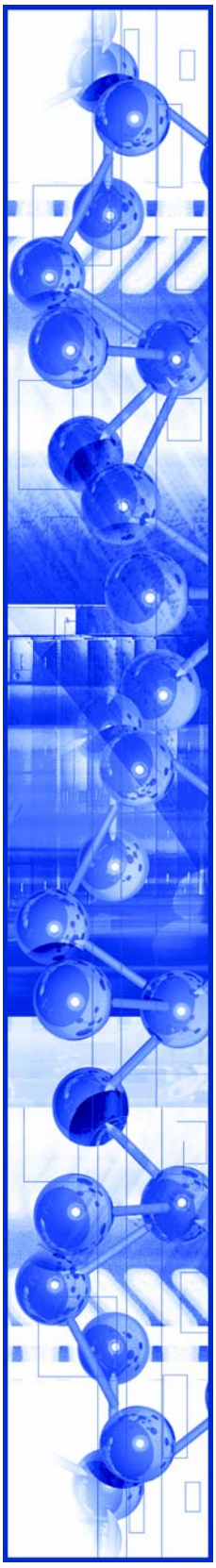

Vantage ReView™ Stripping Buffer

A ready-to-use buffer for removal
of tightly associated antibodies on Western blots

Code	Description	Size
N592-500ML	Vantage ReView™ Stripping Buffer	500 ml

Description:

Vantage ReView™ Stripping Buffer is a robust stripping solution for removing tightly associated primary and secondary antibodies from Western blots prior to reprobing with additional antibodies. Reformulated to eliminate the use of β-mercaptoethanol, it is supplied as an odor-free, ready-to-use solution.

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 derived from different electrophoresis runs. These problems can be eliminated by probing a single blot with multiple antibodies. Unfortunately reagents conventionally used to dissociate strong antigen-antibody complexes require the presence of noxious sulfhydryl reducing reagents to remove bound antibodies. In contrast, Vantage ReView™ Stripping Buffer is an odor-free, ready-to-use stripping buffer that effectively dissociates strong antibody-antigen interactions.

Note: As with other stripping buffers, Vantage ReView™ Stripping Buffer will not dissociate the biotin:avidin interaction. Vantage ReView™ Stripping Buffer will not remove precipitating detection agents.

Storage/Stability:

Vantage ReView™ Stripping Buffer 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:

- 50°C oven
- Fume hood
- Sealed container
- Shaker

***Note**: Blots may be stored in PBS or TBS at 4°C until stripping can be performed.*

***Note**:* Vantage ReView™ Stripping Buffer will not remove precipitating detection agents.

1. Warm Vantage ReView™ Stripping Buffer to 50°C in a fume hood.
2. Rinse the membrane once after ECL or fluorescent detection in Wash Buffer.
3. Submerge blot in a sufficient quantity of Vantage ReView™ Stripping Buffer to completely wet the blot. Approximately 20 ml will cover an 8 x 10 cm blot.
4. Incubate the blot in Vantage ReView™ Stripping Buffer for approximately 30 minutes at 50°C **in a sealed container, or in a fume hood, with gentle shaking.**

***Note:** For best results, Incubation time and temperature should be optimized or empirically determined for each antibody. The time and temperature of incubation depend on the interaction between antibodies and proteins, strong interactions need higher temperatures or longer incubations.*

5. Remove the blot from Vantage ReView™ Stripping 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.
      - 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
N217-100ML	Visiglo™ AP Plus 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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