Gentle ReView™ Stripping Buffer

Code	Description	Size
N552-1L	**Gentle ReView™ Stripping Buffer**	1 L
N552-500ML	**Gentle ReView™ Stripping Buffer**	500 mL
N552-15ML	**Gentle ReView™ Stripping Buffer**	15 mL
N552-15ML-SAMPLE	**Gentle ReView™ Stripping Buffer**	15 mL

General Information

VWR Life Science AMRESCO's Gentle ReView™ Stripping 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 re-probe 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™ Stripping Buffer. However, incubation times and temperatures are dependent upon the affinity of the antibody-antigen interaction and may need to be optimized for each combination used.

- Odor-free, room temperature procedure
- Minimize loss of antigen

Storage/Stability

This solution is stable for at least one (1) year when stored cold (2 – 8°C).

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Required Materials Not Supplied

- Western blot that has been blocked, probed, and detected with a chemiluminescent substrate.
- Shaker
- Wash buffer, such as TBS (J640) or PBS (E404) supplemented with 0.05% Tween (0777)
- Primary and secondary antibodies
- X-ray film or digital imaging system for detection of chemiluminescence

Protocol/Procedure

Notes:

- As with other stripping buffers, Gentle ReView™ Stripping Buffer for Western blots will not dissociate the biotin-avidin interaction.
- Gentle ReView™ Buffer will not remove precipitating detection substrates.
- If circumstances do not allow for the immediate stripping of the blot, store it at 4°C in 1X PBS.
- The blot may be stripped and re-probed several times. Subsequent probings may have reduced signal if the antigen is labile or stripping has damaged the antigen on the blot.

1. Prior to use, equilibrate the Gentle ReView™ Stripping Buffer solution to room temperature.
2. After chemiluminescent detection of proteins on the Western blot, gently rinse the blot in wash buffer.
3. Submerge the blot in a sufficient quantity of Gentle ReView™ Stripping Buffer to completely wet the blot and allow free movement of the blot during gentle agitation. At least 10 mL of Gentle ReView™ Stripping 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™ Stripping Buffer 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 37ºC, 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 empirically optimized for each antibody.*
5. Remove the blot from Gentle ReView™ Stripping Buffer solution, and wash several times in wash buffer.

Note: *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.*

6. Testing for secondary antibody removal:
    a. Incubate the blot with a freshly prepared working solution of chemiluminescent reagent, such as VisiGlo™ Select HRP Chemiluminescent Substrate Kit (1B1583).
    b. Expose the blot to film or CCD camera for 5 minutes. No signal should be detected.
        i. If a signal is present repeat step 3 for a longer incubation time. Incubation can be performed at higher temperatures 30 – 37º C, if required.
        ii. If no signal is detected, continue with step 7.
7. Test for primary antibody removal:
    a. Incubate the blot with the enzyme-conjugated secondary antibody and then wash the blot and incubate in a freshly prepared working solution of chemiluminescent reagent according to usual procedure.
    b. Expose the blot to film or CCD camera for 5 minutes. No signal should be detected.
        i. If a signal is detected return to step 3 and strip the blot for an additional 5 – 10 minutes. Retest for signal before re-probing.
        ii. If no signal is detected, the blot has been successfully stripped and is ready for another round of immunoblotting.

    Note: ***The blot must be re-blocked before beginning new round of probing.***

References

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

Directions for Use

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ZY0572
Rev. 3 02/2016