

OneMinute[®]Plus Western Blot Stripping Buffer^{*}

Cat. No. GM6011 GM6012 GM6015

2008.10

Number Description

GM6011	<i>OneMinute[®]Plus Western Blot Stripping Buffer (125ml)</i>	25 Tests
GM6012	<i>OneMinute[®]Plus Western Blot Stripping Buffer (20ml)</i>	4 Tests
GM6015	<i>OneMinute[®]Plus Western Blot Stripping Buffer (500ml)</i>	100 Tests

Storage: Store at room temperature. Please refer to label for the Expiration date.

Product shipped at ambient temperature.

Introduction

OneMinute[®]Plus Western Blot Stripping Buffer specifically and effectively removes primary and secondary antibodies from Western blots without removing the transferred proteins. It is a robust but gentle solution for stripping Western blots that were detected with chemiluminescent substrates. It allows the use of a single membrane for multiple times of re-probes.

Distinct advantages of *OneMinute[®]Plus Western Blot Stripping Buffer*:

- Stripping is finished and membrane is ready for re-probing with primary antibody in seconds (~1min)
- Multiple times of re-probes (~10 times)
- Re-blocking of blots is usually not necessary
- Antibody removal is done at room temperature. No heating is required
- Long shelf life

Additional material required

- Western blot, previously blocked, probed and detected with a chemiluminescent substrate
- Washing Buffer such as phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) with 0.05% Tween-20.

Protocol

1. **Wash blot in Washing Buffer to remove the chemiluminescent substrate.** Blot may be stored in PBS or TBS at 4°C until the stripping procedure can be performed. NEVER LET THE BLOT DRY.
2. **Place the blot in 5ml *OneMinute[®]Plus Western Blot Stripping Buffer* and shake for 30 sec ~ 1min. Then, add 20ml Washing Buffer, decant the mixture.** Use a sufficient volume to ensure the blot is completely wetted by *OneMinute[®]Plus Western Blot Stripping Buffer* (5ml per test is required for a standard 8 x 10 cm blot).
Note: Optimization of stripping condition is essential for best results. The blot can be treated by *OneMinute[®]Plus Western Blot Stripping Buffer* for longer time (~ 5 min) or at higher temperature (37°C).
3. **Wash the blot in 10~20ml Washing Buffer for 3 times.** Briefly shake in hand for 10~20 seconds each time.

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4. (Optional) Test for removal of the antibodies as follows:

Test for complete removal of secondary antibody: incubate the blot with new chemiluminescent substrate and expose the blot to film. If no signal is detected using a 5-minute exposure, the secondary antibody has been successfully removed.

Test for complete removal of the primary antibody: incubate the blot with secondary antibody, then wash it in washing buffer. Incubate the blot with new chemiluminescent substrate and expose the blot to film. If no signal is detected with a 5-minute exposure, the primary antibody has been successfully removed.

5. The blot is ready for subsequent re-probing. Perform the re-probing with primary antibody in proper buffer containing 1~5% milk. Re-blocking the blot is usually not necessary but might be required in some cases.**Notes:**

- Be sure to keep the Western blot wet all the time. Never let the blot dry.
- *OneMinute[®] Plus* Western Blot Stripping Buffer will not dissociate interaction between biotin and avidin.
- Stripping and re-probing fluorescent Western blots is not recommended because unexpected background is generated.

Troubleshooting:

Problem	Possible Cause	Solution
Background in subsequent detection	Antibody concentrations are too high	Strip again and dilute antibody concentration
	Not sufficiently blocked after stripping	Strip again, re-block with 5% milk before re-probing
Signals obtained after stripping	Extremely high-affinity antigen-antibody interaction	Increase stripping time and temperature
		Use primary antibody from different host in subsequent re-probing
	High-abundance of antigen	Use more dilute antibody concentration and less probing time
		Blot to low-abundance antigen first, then strip and re-probe with high-abundance antigen
	Chemifluorescent signals were detected	Use X-ray film to detect signal. If X-ray film is not available, make sure only chemiluminescent signals are detected. Some WB detection reagents, such as ECL-Plus, generate both chemiluminescent and chemifluorescent signals.
The blot was dried	Prepare a new blot. Never let the membrane dry.	
Loss of signal or no signal after stripping and re-probing	Antigen is in low abundance or not present	Prepare a new blot and probe for low-abundant antigens first
		Load more protein in the gel
	Antibody concentrations too low	Increase antibody concentrations

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* US Patent Pending