

OneMinute[®] Western Blot Stripping Buffer*

Cat. No. GM6001 GM6002

2008.10

Number Description

GM6001 *OneMinute*[®] Western Blot Stripping Buffer (125ml) 25 TestsGM6002 *OneMinute*[®] Western Blot Stripping Buffer (20ml) 4 Tests**Storage:** Store at room temperature. Please refer to label for the Expiration date.

Product shipped at ambient temperature.

Introduction

OneMinute[®] 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*[®] 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

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*[®] 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*[®] 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*[®] 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.
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

OneMinute[®] Western Blot Stripping Buffer^{*}**Cat. No. GM6001 GM6002**

2008.10

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[®]* 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.
	High sensitive WB detection reagents used in subsequent re-probing	Keep using same WB detection reagents all the time. Detect weak signals first, then strip and detect strong signals in subsequent re-probing.
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

This product is for research use only and not for human or diagnosis use. The warranty limits our liability only to replacement of the product. There is no obligation to replace this product as the result of accident, disaster, misuse, fault, negligence, improper storage or handling of the products. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by GM Biosciences Inc. We shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use the product.

* US Patent Pending