INSTRUCTIONS **GM Biosciences**

OneMinute®Advance Western Blot Stripping Buffer*

Cat. No. GM6031

2008.10

Number Description

GM6031 ***OneMinute®Advance* Western Blot Stripping Buffer 25 Tests**

Contents: 5ml 25X Supplement (GM6021, Buffer A, **Store at 4 ºC**)
125ml *OneMinute®* WB Stripping Enhancing Buffer (GM6021, Buffer B, **Store at RT**)
125ml *OneMinute®* Western Blot Stripping Buffer (GM6001, **Store at RT**)

Please refer to label for the Expiration Date. Product shipped at ambient temperature.

Introduction

***OneMinute®Advance* Western Blot Stripping Buffer** is harsh to antibody, but gentle to transferred protein. It specifically and effectively removes primary and secondary antibodies from Western blot without removing the transferred protein, which was detected with a chemiluminescent substrate. It allows the use of a single membrane for multiple times of re-probes, and is designed especially for high-affinity antibody. ***OneMinute®Advance* Western Blot Stripping Buffer** represents the world-best WB stripping reagents.

Advantages:

- Stripping high-affinity antibody
- Blot is ready for re-probing in minutes
- Multiple times of re-probes (~10 times)
- Re-blocking is usually not necessary

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. **Dilute 25X Buffer A into Buffer B to make 1X working solution.** For example, dilute 1ml 25X Buffer A into 24ml Buffer B to give 25ml 1X working solution. 5ml 1X working solution is enough for a standard Western blot (8 x 10 cm blot).
   Note: Freshly made 1X working solution is recommended to obtain best results. The 1X working solution is stable for only 2 weeks at room temperature.
3. **Place the blot in 5ml 1X working solution made in step 2, shake for 5min at room temperature.** Use sufficient volume to ensure the blot is completely wetted by the working solution (5ml per test is required for a standard 8 x 10 cm blot).
4. **Decant working solution, add *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

GM Biosciences, Inc.
5350 Partners Ct., Suite C
Frederick, MD 21703 USA
Tel: 240-595-9177
Fax: 301-378-2862
www.gmbiosciences.com

OneMinute®*Advance* Western Blot Stripping Buffer*

Cat. No. GM6031

2008.10

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 with *OneMinute*® Western Blot Stripping Buffer for longer time (~ 5 min) or at higher temperature (37ºC).

5. **Wash the blot in 10~20ml Washing Buffer for 3 times.** Briefly shake in hand for 10~20 seconds each time.
6. **(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.
7. **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:

- *OneMinute*®*Advance* Western Blot Stripping Buffer will not dissociate interaction between biotin and avidin.
- Stripping fluorescent Western blots is not recommended because unexpected background is generated.
- Freshly made 1X working solution is recommended to obtain best results. 1X working solution is stable for only 2 weeks at RT.

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

* US Patent Pending

GM Biosciences, Inc.
5350 Partners Ct., Suite C
Frederick, MD 21703 USA
Tel: 240-595-9177
Fax: 301-378-2862
www.gmbiosciences.com