

OneMinute[®] WB Stripping Enhancing Buffer*

Cat. No. GM6021

2008.10

Number Description

GM6021 *OneMinute*[®] WB Stripping Enhancing Buffer **25 Tests**
(Buffer A: 25X Supplement 5ml ; **Storage: Store at 4 °C**)
(Buffer B: *OneMinute*[®] WB Stripping Enhancing Buffer 125ml; **Storage: Store at room temperature**)

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

Introduction

Pretreatment of Western blots with *OneMinute*[®] WB Stripping Enhancing Buffer significantly enhances the efficiency of Western Blot stripping reagents (such as *OneMinute*[®] Western Blot Stripping Buffer). Transferred protein on the blot keeps intact after treating with *OneMinute*[®] WB Stripping Enhancing Buffer. The combination of this buffer with *OneMinute*[®] Western Blot Stripping Buffer creates the world-best stripping reagents, especially for high-affinity antibody.

Additional material required

- Western Blot stripping reagents, such as *OneMinute*[®] Western Blot Stripping Buffer (GM6001, GM6002)
- 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. Decant working solution.** 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. **Add *OneMinute*[®] Western Blot Stripping Buffer and shake for 30 sec ~ 1min. Then, add 20ml Washing Buffer and 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 with *OneMinute*[®]

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Western Blot Stripping Buffer for longer time (~ 5 min) or at higher temperature (37°C).

5. **Wash the blot in 10~20ml Washing Buffer for 3 times.** Briefly shake in hand for 10~20 seconds each time.
6. **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.
- Freshly made 1X working solution is recommended to obtain best results. The 1X working solution is stable for only 2 weeks at room temperature.
- Stripping and re-probing fluorescent Western blots is not recommended because unexpected background is generated.

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* US Patent Pending