Multi-Western™

Stripping Buffer

Catalog Number	Description	Size
SB01-01	Multi-Western™ stripping buffer (10x)	100 ml
SB02-01	Multi-Western™ stripping buffer (1x)	500 ml

Introduction

In practice, a single Western blot membrane (PVDF or nitrocellulose) can be used to investigate multiple protein targets. The advantages include: (1) It saves samples, reagents and time; (2) Re-using the same blot ensures the reliable comparison among different targets, since exact reproducibility of the protein pattern by running and blotting multiple gels is difficult. Therefore, efficient stripping, i.e. removal of primary and secondary antibodies from a Western blot membrane, becomes important and necessary.

The Multi-Western™ Striping Buffer contains special reagents, which provide a quick and effective method to remove primary and secondary antibodies from membrane to enable several re-probings on the same membrane.

The features of the Multi-Western™ Striping Buffer include:

- Membrane stripping is done at room temperature. No heating is needed.
- Stripping only takes 5 - 15 minutes. Membrane can be re-probed in 25 minutes.
- Stripping strength is adjustable by different dilutions.
- No pungent-smell β-mercaptoethanol is added in the buffer.

Storage

- Stripping buffer is shipped at ambient temperature.
- It should be stored at room temperature or 4°C upon arrival.
- The product is stable for 1 year after receipt.
- If stripping buffer crystallizes upon storage, warm at 37°C water bath to dissolve before use.
- Make sure the cap is tight during storage.

Caution

- Always wear gloves during Western Blot stripping. If the skin or body parts contact with the reagent, wash and rinse with large amount of water immediately.
- For research use only.

Stripping Procedure

Note: Membranes that cannot be stripped immediately after chemiluminescent detection may be stored in phosphate buffered saline (PBS) at 4°C until the stripping procedure can be performed.

1. **Dilute 10x Stripping Buffer (SB01-01) with distilled water to obtain a 1x solution. If using 1x Stripping buffer (SB02-01), go to Step 2 directly.** If crystals formed in the Stripping Buffer, warm at 37°C water bath until it is dissolved completely. For antibodies with strong signals or 1x solution is not sufficient, dilute Stripping Buffer (10x) to 1.5x.

2. **Rinse the membrane with distilled water once. Put the membrane into the container with 1x Stripping Buffer. Incubate with gentle shaking for 5-10 minutes at room temperature.** Use a sufficient volume to ensure that the blot is completely wetted (i.e., approximately 10 ml required for an 8 x 10 cm blot).

   Incubation time: In general, 10 minutes will be enough for most of the antibodies. If high affinity antibodies are used, increase the incubation time to 20 minutes. ***Don't exceed 30 minutes.***

3. **Remove the Stripping Buffer and rinse the membrane 3 times with distilled water.**

4. (Optional): **Test for complete removal of the HRP label (e.g., secondary antibody): Incubate the membrane with new ECL solution and expose to film.**
   - If no signal is detected using a 5-minute exposure, the HRP conjugate has been successfully removed from the antigen or primary antibody.
   - If signal is detected with either test in step 4, return to step 2, stripping for an additional 5-15 minutes.

5. **Place the membrane into a clean container with Western Blot blocking buffer. Incubate for 10 minutes. The membrane is now ready for next antibody probing.**

Notes:

- Membrane may be stripped and re-probed several times. Subsequent reprobings may result in decreased signal if the antigen is labile in Multi-Western™ Stripping Buffer.
- Re-blocking a membrane is usually not necessary after stripping but may be required in some applications.
- PVDF membrane is highly recommended to minimize loss of sample proteins.
- Chemiluminescent reagents such as ECL are recommended as they will not leave a stain and are more sensitive than colorimetric reagents.