Cat # SL100324 Store at 4 ~ 23 $^0$C

# BlotFresh™ Western Blot Stripping Reagent

☐ **Small** **125 ml**

☐ **Large** **500 ml**

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**This product is for laboratory research ONLY and not for diagnostic use**

## Description:

BlotFresh™ Western Blot Stripping Reagent is formulated to be effective for removal of antibodies from Western blots that have been developed with chemiluminescence or radioactive iodine or other isotopes. The membrane can be nitrocellulose or PVDF/nylon. The stripped membrane is OK for re-probing as that of regular western blot and for mass spectrometry.

## Reuse of blots offers many advantages:

- Effective use of samples that are available in limited amounts
- Comparison of images obtained with different antibodies in the same blot
- Confirmation of results with the same or different antibodies
- It is simply more economical and less time consuming to reuse blots for re-probing and mass spectrometry

## Instructions:

### Application I: For re-probing

After initial probing, be sure to keep membrane wet in TBST buffer in fridge. NEVER LET THE BLOT DRY!

1. Pour 15~30 ml stripping reagent to a clean container and put the blot in the container. Make sure that the blot is fully submerged with the stripping buffer.
2. Incubate the blot in stripping reagent at room temperature for 5~15 minutes with strong agitation. Though incubation with the high affinity antibodies need to be optimized, 15 minutes stripping at room temperature is usually sufficient for most of antibodies.

**Note:** Optimization of both incubation time and temperature is essential for best results. In general, higher affinity antibodies will require at least 15 minutes of stripping and may require an incubation temperature of 37 $^0$C .

3. Wash for 2x5 minutes in TBS-T at room temperature using large volumes (e.g. 100 ml) of wash buffer.

**Note:** To test the stripping effect, pour ECL reagent on blot followed by 5 minutes exposure to a film.

4. Block blot with TBS-T buffer with 5% defat milk powder for 45 minutes at room temperature.
5. Immunodetection as normal.

### Application II: For Mass Spectrometry

After initial probing, be sure to keep membrane wet in TBST buffer in fridge. NEVER LET THE BLOT DRY!

1. Pour 15~30 ml stripping reagent to a clean container and put the blot in the container. Make sure that the blot is fully submerged with the stripping buffer.
2. Incubate the blot in stripping reagent at room temperature for 15~30 minutes with strong agitation. Though incubation with the high affinity antibodies need to be optimized, 15 minutes stripping at room temperature is usually sufficient for most of antibodies.

**Note:** Optimization of both incubation time and temperature is essential for best results. In general, higher affinity antibodies will require at least 15 minutes of stripping and may require an incubation temperature of 37 $^0$C.

3. Wash for 2x5 minutes in TBS-T at room temperature using large volumes (e.g. 100 ml) of wash buffer.

**Note:** To test the stripping effect, pour ECL reagent on blot followed by 5 minutes exposure to a film.

4. Locate the interested protein by comparing the stripped blot with the exposed film and cut the interested band.
5. Digest the cut band followed by mass spectrometry.

## References:

1. Kaufmann, S.H., et al. (1987). Anal. Biochem. 161, 89-95.
2. Kaufmann, S.H. and Kellner, U. (1998). Erasure of Western blots after autoradiographic or chemiluminescent detection. In Immunochemical Protocols. Ed. Pound, J.D. Humana Press, Totowa, NJ, 223-235
3. Schrager, J.A., et al. (2002). J. Biol. Chem. 277, 6137-6142

**Storage:** Upon arrival store this product at 4 $^0$C ~room temperature. For long term, store the product at 4 $^0$C. Product shipped at ambient temperature.