Western Stripping Buffer (Weak alkali)

Introduction

Western Blot Stripping Buffer is used for the reuse of membranes onto which proteins have been transferred during Western blotting. After completing primary and secondary antibody binding and subsequent chemiluminescent detection, it is sometimes necessary to detect reference proteins with relatively stable expression levels, such as tubulin or actin, or to detect other proteins for comparison. By using this stripping buffer to thoroughly remove the primary and secondary antibodies, the used membrane can be conveniently reused to detect other proteins. Compared to running a new SDS-PAGE gel, this method is not only time-saving and labor-saving but also eliminates errors caused by reloading samples, thereby improving comparability.

Reusing the same membrane multiple times with Western Blot Stripping Buffer may lead to a reduction in protein signal. However, this product has been tested with various antibodies and typically allows for 3–5 reuses of the membrane.

This reagent takes only approximately 20–30 minutes to enable membrane reuse, after which you can proceed with blocking and subsequent Western blotting steps.

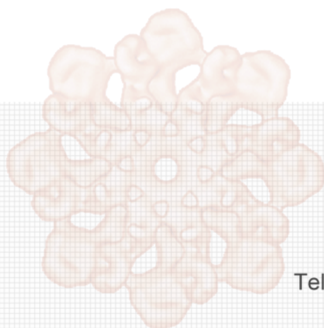
Different Western blot stripping buffers vary slightly in their principles for removing bound primary and secondary antibodies. This product utilizes different combinations of detergents, acidic agents, alkaline agents, reducing agents, chelators, salt concentrations, and other specialized reagents to disrupt primary and secondary antibody binding without causing significant loss of the proteins transferred onto the membrane.

Protocol

1. After completing Western chemiluminescent detection, rinse the membrane in distilled water for 5 minutes.
2. Discard the distilled water and add an appropriate amount of Western Stripping Buffer (Weak alkali), ensuring the membrane is fully covered. Shake on an orbital shaker for 30–60 minutes.
3. Discard the Western Stripping Buffer (Weak alkali) and remove any residual liquid. Rinse the membrane 3–4 times with TBS, TBST, or PBS, shaking for 3–5 minutes each time on an orbital shaker.
4. Proceed with blocking and subsequent Western blot steps.

Note

1. When using horseradish peroxidase (HRP), any blocking step should be performed with 5% non-fat milk or BSA. If using alkaline phosphatase, any blocking step should be performed with casein.
2. When stored at 4°C or -20°C, this product may appear light yellow to pale pink. After standing at room temperature for a while, it will gradually become colorless. Upon returning to low-temperature storage, it will gradually turn light yellow to pale pink again. This color change is a normal phenomenon for this product. Testing has confirmed that the product performs identically regardless of whether it is colored or colorless.
3. For best results, PVDF membranes are recommended. However, nitrocellulose membranes can also be used with this stripping buffer.
4. This reagent is suitable for Western detection using ECL chemiluminescent reagents. It is not suitable for Western detection using non-chemiluminescent methods such as DAB or NBT/BCIP.
5. This stripping buffer is mildly corrosive. Please take appropriate precautions during use.
6. Storage conditions: Store at 4°C; stable for one year. For long-term storage when not in frequent use, can be stored at -20°C.
7. This product is for scientific use only.



APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com