The Celltechgen™ Mammalian Protein Extraction Reagent

CTG-PA0014-A	The Celltechgen™ Western Blot Stripping Buffer,250ml	ISSUE DATE 9 July 2019
CTG-PA0014-B	The Celltechgen™ Western Blot Stripping Buffer,500ml	ISSUE DATE 9 July 2019

Storage: Upon receipt store kit components at 4°C. Product is shipped at ambient temperature.

Introduction

Nitrocellulose and PVDF membranes probed by Western blotting procedures and detected by chemiluminescent or other non-precipitating substrates can be stripped and re-probed using the Celltechgen™ Western Blot Stripping Buffer. Western blotting is widely used to detect and compare proteins in complex mixtures, and chemiluminescence has largely replaced chromogenic substrates as the most convenient and sensitive method of detection. One advantage of chemiluminescence is the ability to strip and reprobe the protein mixture on the membrane. Traditional stripping methods use conditions that are effective for only low-affinity antibody-antigen interactions or are so harsh that they tend to adversely alter the antigen for subsequent immunoprobing. The Celltechgen™ Western Blot Stripping Buffer is a robust but gentle formulation for stripping primary and secondary antibodies from blots to enable several reprobings on the same membrane. The Celltechgen™ Western Blot Stripping Buffer is ideal for use with West Chemiluminescent Substrates.

Additional Materials Required

• Western blot, previously blocked, probed and detected with chemiluminescent (i.e., nonprecipitating) substrate
• Wash buffer such as Tris-buffered saline (TBS) or phosphate-buffered saline (PBS) with 0.05% Tween 20
• Primary and secondary antibodies for both first and second Western blotting experiments

Procedure for Stripping an Immunoblot

Notes:

• Blots may be stored in PBS or TBS at 4°C until the stripping procedure can be performed.
• Restore Western Blot Stripping Buffer will not dissociate interactions between a biotinylated target protein and avidin-conjugated probes.
• Stripping and reprobing fluorescent Western blots is not recommended because results are typically inconsistent.

1. Warm the bottle of Restore Western Blot Stripping Buffer to room temperature.
2. Place the blot in Restore Western Blot Stripping Buffer and incubate for 5 to 15 minutes at 37°C. Use a sufficient volume to ensure that the blot is completely wetted (i.e., ~20mL required for an 8 × 10cm blot).

Note: Optimization of both incubation time and temperature is essential for best results. For some antibodies, room temperature incubation is sufficient. However, high-affinity antibodies or saturated blots (excess secondary antibody) may require incubation for an additional 5 to 10 minutes at 37°C.

3. Remove the blot from the Restore Western Blot Stripping Buffer and wash in TBS or PBS.
4. Test for the removal of the immunodetection reagents as follows:

• Test for complete removal of the HRP label (e.g., secondary antibody): Incubate the membrane with new TopSignal West Working 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.
• Test for complete removal of the primary antibody: Incubate the membrane with the HRP-labeled secondary antibody, followed by a wash in wash buffer. Incubate in new TopSignal West Working Solution and expose to film. If no signal is

detected with a 5-minute exposure, the primary antibody has been successfully removed from the antigen.

5. If signal is detected with either test in step 4, return to step 2, stripping for an additional 5-15 minutes. Some antigen/antibody systems require increased temperature and/or longer incubation times to strip them fully. Optimize stripping time and temperature to ensure complete removal of antibodies while preventing damage to the antigen.

6. After determining that the membrane is properly stripped, the second immunoprobing experiment may be performed.

Notes:

• Blot can be stripped and reprobed several times but might require longer exposure times or a more sensitive chemiluminescent substrate. Subsequent reprobings might result in decreased signal if the antigen is labile in Restore Western Blot Stripping Buffer. Analysis of the individual system is required.

• Reblocking the membrane is recommended after stripping.