Signal clear stripping buffer

WESTERN BLOTTING REAGENTS

Stripping and reprobing is a critical step in Western blotting when researchers need to detect multiple protein targets on the same membrane after initial enhanced chemiluminescence (ECL) detection. Rather than repeating the entire workflow from sample preparation, stripping removes bound primary and secondary antibodies so the membrane can be reprobed with a different antibody combination, generating additional datasets from a single experiment.

Key benefits:

- **Ready-to-use formulation;** requires no mixing, heating, or preparation, saving time and removing variability so that stripping is straightforward.
- **Gentle formulation that preserves immobilized proteins;** efficiently removes primary and secondary antibodies without detectable protein damage under the tested conditions, enabling reprobing of the same membrane for up to four or more cycles.
- **No reblocking required after stripping;** eliminates an extra workflow step, providing faster turnaround from stripping to reprobing by up to 2 h per membrane.
- **Formulation contains no β-mercaptoethanol** and is designed to minimize laboratory odor.
- **No reblocking after stripping is required,** enabling faster reprobing while maintaining signal.
- **Protects precious samples** such as primary patient biopsies, rare cell lines, and limited clinical material by maximizing the data generated from every membrane.



Fig 1. Contents of Signal clear stripping buffer.

Traditional stripping buffers often rely on harsh reducing agents such as β-mercaptoethanol, and require heating from 50°C to 70°C in a water bath, extended washing, and additional reblocking steps before reprobing. These conditions can damage immobilized proteins, reduce signal quality on subsequent probes, and generate strong odors that can be hazardous and uncomfortable in shared laboratory environments. As a result, many researchers avoid stripping altogether and instead rerun the entire Western Blot, risking the loss of around 3 d of work, valuable samples, and reagents.

Signal clear stripping buffer is a ready-to-use solution designed to efficiently remove both primary and secondary antibodies without damaging the immobilized proteins. Our stripping buffer requires no heating, mixing, or reblocking under the recommended protocol. The workflow is simple: strip, wash, and reprobe, enabling researchers to generate additional data from a single blot while reducing time, sample consumption, and effort associated with repeating the workflow.

Applications

Stripping and reprobing is used to maximize data output when samples are limited or when multiple targets must be analyzed on the same membrane. Signal clear stripping buffer supports a range of applications, including:

- Detection of multiple protein targets on a single membrane to conserve precious sample material.
- Sequential probing for loading controls (e.g., GAPDH, β-actin, β-tubulin) after detection of the target protein.
- Reprobing with phosphospecific and total protein antibodies on the same membrane.
- Maximizing data from clinical samples, patient biopsies, or rare cell lines where sample volume is limited.
- Standardizing stripping protocols in core facilities and shared laboratories where multiple users require a consistent, reliable method.

Stripping efficiency

Effective stripping depends on two complementary requirements: Detachment of bound primary and secondary antibodies; and retention of the immobilized antigen so that the epitope remains available for accurate reprobing.

To assess both, we ran A-431 cell lysate (Abcam) on SDS-PAGE, transferred to a nitrocellulose (NC) membrane, and probed for GAPDH. After initial ECL detection (Fig 2.A), the membrane was incubated with our Signal clear stripping buffer for 20 min at room temperature on a rotating platform, then washed in PBS-T for 5 min.

To quantitate residual bound antibody, we reincubated the stripped membrane with the HRP-conjugated secondary antibody alone and re-imaged (Fig 2.B). Any remaining signal at the GAPDH molecular weight indicates incomplete removal of the original antibody complex.

Following the secondary antibody-only reprobe, the residual signal was 0.1% of the original band intensity under the test conditions evaluated, confirming near-quantitative removal of the antibody complex. The residual signal was less than 0.1%, well below the design specification of <1%, showing that the stripping buffer efficiently removed both primary and secondary antibodies in a single 20 min step without heating or reducing agents.

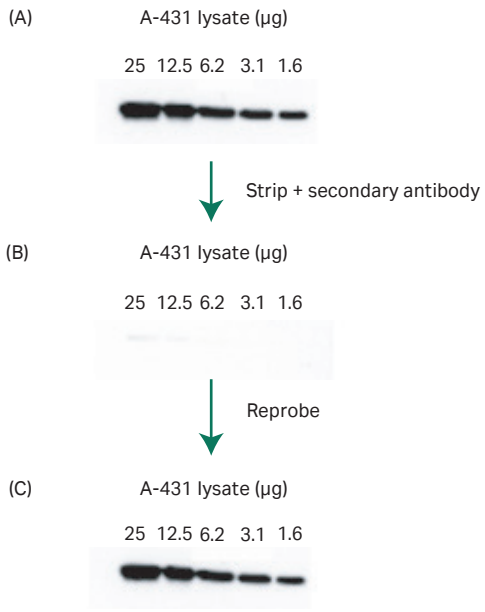


Fig 2. Western Blot images showing (A) initial probing for GAPDH, (B) post-stripping secondary antibody-only reprobe confirming near-complete antibody removal, (C) full reprobe with primary and secondary antibodies. All blots used our Easy tab ECL prime tablets and Amersham™ ImageQuant™ 800 for detection.

When we reprobed the stripped membrane with fresh primary and secondary antibodies under identical exposure and contrast settings (Fig 2.C), the recovered signal reached 102% of the original band intensity (Table 1). Full recovery suggests the epitope is preserved and that stripping does not displace the target protein from the membrane.

Signal clear stripping buffer requires no additional blocking step before reprobing. The increase in signal compared to the initial reading, may indicate that the stripping buffer has removed blocking proteins that partially occluded the epitope of the target protein during the first detection step. As a result, the reprobe antibody may have improved access to the target, leading to an increase in detectable signal.

Table 1. Stripping efficiency results. Data generated using Amersham ImageQuant 800 imaging system

Parameter	Result
Stripping conditions	20 min, room temperature, rotating platform
Residual signal after stripping (secondary antibody-only reprobe)	0.1% of original signal
Signal after reprobe	102% from original signal

Preserving the immobilized antigen is as important as removing the antibody, since the target must remain on the membrane for the reprobe to be quantitative. Signal clear stripping buffer is formulated to retain more than 70% of protein on the membrane under standard stripping conditions. The full signal recovery reported in Table 1 confirms that the epitope is preserved through the strip and reprobe cycle.

Equivalent stripping efficiency and protein retention were obtained on Amersham Protran premium 0,45µm NC, confirming that performance is maintained across PVDF supports.

Reprobing performance

A key measure of a stripping buffer is whether the immobilized antigen withstands repeated stripping and reprobing without progressive loss of signal. To test this, we probed a single membrane carrying a two-fold serial dilution of A-431 cell lysate for GAPDH and then subjected it to four sequential strip and reprobe cycles with Signal clear stripping buffer (Fig 3).

A secondary antibody-only reprobe after the first strip confirmed complete removal of the primary antibody, and each subsequent reprobe with fresh primary and secondary antibody reproduced the expected dilution series with comparable band intensity and no visible degradation of the signal. Consistent detection across cycles, even at low lysate loads, means that the target protein remained membrane-bound and antigen integrity was maintained through at least four reprobing cycles.

Reprobing enables the analysis of multiple target proteins on a single membrane, maximizing the data generated from each experiment. Reprobing multiple targets on the same membrane means fewer reruns, making the most of limited samples, lower reagent and consumable costs.

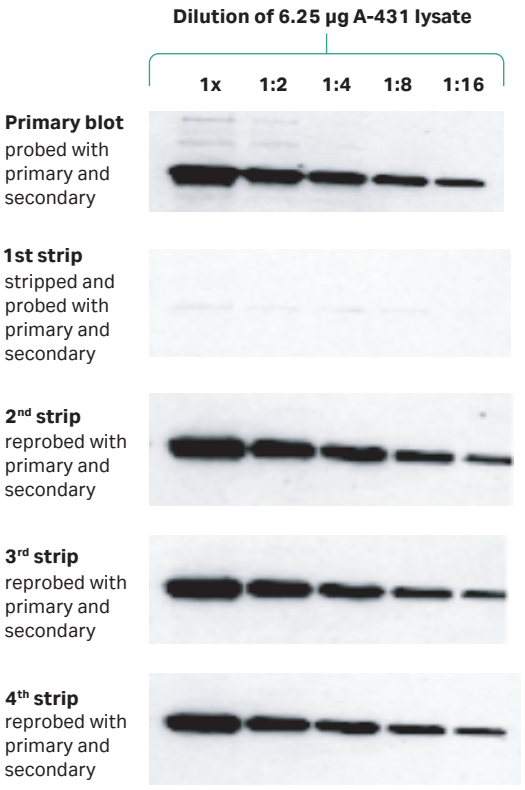


Fig 3. Consistent GAPDH detection after repeated stripping and reprobing of a single membrane. A two-fold serial dilution of A-431 cell lysate (6.25 µg) was probed for GAPDH. Band intensity is maintained across every cycle, including at the lowest sample loads, demonstrating that Signal clear stripping buffer removes bound antibody efficiently while preserving the immobilized antigen and its sensitivity through repeated strip and reprobe cycles.

Background signal stability

The value of a reprobed blot depends not only on the strength of the specific band but on its signal-to-noise ratio. Aggressive stripping methods, strips blocking completely exposing reactive sites on the membrane, allowing the next set of antibodies to bind nonspecifically and increase background noise.

Signal clear stripping buffer is designed to remove bound antibody without stripping away the underlying block or altering the membrane surface, so no new sites are created for nonspecific binding on the subsequent probe.

Membranes reprobed after stripping showed no measurable rise in background noise relative to the initial blot, so the signal-to-noise ratio was maintained from one cycle to the next. This stable background is what allows faint and low-abundance targets to be distinguished reliably even after several rounds of stripping and reprobing.

Head-to-head comparison with traditional methods

Signal clear stripping buffer offers significant workflow, safety, and performance advantages over traditional homemade stripping buffers and other commercially available alternatives. In Table 2, we compare key parameters based on published product specifications and standard laboratory practice.

Table 2. Comparison of stripping methods

Parameter	stripping buffer (homemade)	ThermoFisher, Restore	Merck-Millipore, ReBlot	Cytiva, signal clear stripping buffer
Preparation	Requires reagent sourcing and mixing	Ready to use	Requires dilution (10×)	Ready to use
Heating required	Yes (50°C to 70°C water bath)	Often recommended	Often required	None (room temperature)
Odor	Strong (β-mercaptoethanol)	Mild	Variable	None
Fume hood required	Yes	Recommended	Recommended	No
Reblocking required	Yes	Yes	Yes	No
Reliable reprobe cycles	1 to 2	2 to 3	1 to 2	4 or more demonstrated in internal testing
Total time saved per membrane	Baseline	Moderate	Moderate	1 to 2 h under typical workflow conditions
Stripping time	30 to 60 min	15 to 30 min	15 to 30 min	20 min

*Competitor data sourced from publicly available product datasheets and specifications.

Key differentiators

1. **Simplicity:** Among the products evaluated in this comparison, Signal clear stripping buffer required no preparation, heating, or reblocking. The entire stripping-to-reprobing workflow can be completed in approximately 25 min at room temperature.

2. **Safety and comfort:** Signal clear stripping buffer contains no β-mercaptoethanol and is designed to minimize laboratory odor. No fume hood needed when used under the recommended protocol, making it suitable for shared or open-plan laboratory environments, and institutional settings where environmental health and safety (EHS) compliance is a priority.

3. **Sample protection:** With four or more reliable reprobing cycles and 102% signal recovery demonstrated in testing, Signal clear stripping buffer enables you to get more data from every membrane. This is particularly important for irreplaceable samples such as primary patient biopsies, rare cell lines, and limited clinical material.

Protocol

Signal clear stripping buffer is designed for a simple, three-step workflow that can be completed in approximately 25 min at room temperature:

1. **Strip:** Incubate membrane in Signal clear stripping buffer (25 mL) for 20 min at room temperature on a rotating platform.
2. **Wash:** Wash membrane in PBS-T buffer for 5 min.
3. **Reprobe:** Proceed directly to primary antibody incubation (no reblocking required).

The entire stripping-to-reprobing process saves 1 to 2 h per membrane compared to traditional methods that require heating, extended washing, and reblocking.

Depending on the target protein and antibody combination used, the stripping protocol may benefit from minor optimization of incubation time. For most standard applications, the 20 min room temperature protocol provides excellent results.

Membrane compatibility

Signal clear stripping buffer has been tested and validated on both NC and PVDF membranes, including Amersham Protran™ NC and PVDF membranes. Excellent stripping efficiency and protein preservation were confirmed on both membrane types.

'Lot-to-lot' consistency

Lot-to-lot consistency has been confirmed through testing of multiple production lots, ensuring reproducible stripping performance across different batches. A certificate of conformity is available for each lot, providing traceability for regulated and publication-quality workflows.

Sustainability designed in

We are committed to advancing sustainable laboratory practices and reducing the environmental impact of routine research workflows.

We provide Signal clear stripping buffer in a 250 mL plastic bottle packaged in a recyclable paper box. To minimize paper usage, the instructions for use are provided in digital format, accessible by scanning the QR code on the product label. The formulation eliminates the need for β-mercaptoethanol, a hazardous reducing agent that presents disposal, safety, and environmental health challenges in laboratory settings.

By enabling four or more reprobing cycles from a single membrane, Signal clear stripping buffer may help reduce overall consumable waste by enabling multiple reprobing cycles from a single membrane. Fewer gels, membranes, antibodies, and detection reagents are consumed when multiple datasets can be extracted from one blot rather than repeating the entire Western Blot workflow. This may help to reduce total reagent consumption, plastic waste, and associated costs.

Technical specifications

Specification	Parameter
Formulation	Ready to use (no mixing, heating, or preparation required)
Stripping time	20 min at room temperature
Residual signal after stripping	< 1% (reprobed with same secondary antibody); 0.1% demonstrated in testing
Protein retention	> 70% retained on membrane after standard stripping
Reprobing signal recovery	> 50% of original signal (100% recovery observed under the conditions tested)
Reprobing cycles	Four or more
Background signal	No increase after stripping
Odor	Odorless (no β-mercaptoethanol)
Membrane compatibility	NC and PVDF
Storage conditions	Room temperature
Shelf life	18 mo at room temperature
Volume per bottle	250 mL (sufficient for approximately 10 blotting membranes, 9 × 8 cm)
Certificate of conformity	Available for each lot

Ordering information

Item	Product code
Cytiva signal clear stripping buffer, 1 × 250 mL bottle	RPN2238

Related products

1. Amersham Hybond and Protran blotting membranes
2. Amersham rainbow molecular weight markers
3. Amersham ECL HRP-conjugated antibodies
4. Easy tab ECL prime tablets
5. Amersham ImageQuant 800 imaging system
6. ImageQuant TL image analysis software

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