

ClearBand Re-Blot Stripping Solution

Troubleshooting Guide

500 ml

Cat No: RBS500

Use this guide for common antibody-stripping problems with ClearBand Re-Blot Stripping Solution. The ready-to-use reagent is intended for removing primary and secondary antibodies from nitrocellulose or PVDF membranes so that the same membrane can be reprobed.

Before You Start - Quick Checks

1. Store at room temperature.
2. Wash away chemiluminescent substrate before stripping.
3. Add 8 μ L beta-mercaptoethanol per 1 mL stripping solution immediately before use.
4. Use 5-10 mL per membrane, incubate for 1 hour at 55°C, then wash with PBS-T or TBS-T for at least 30 minutes.

Problem	Most likely cause	Recommended action
Previous antibody signal remains after stripping	The beta-mercaptoethanol addition, solution volume, temperature, or incubation time may be insufficient.	Use 8 μ L beta-mercaptoethanol per mL, 5-10 mL per membrane, and incubate for 1 hour at 55°C.
Residual chemiluminescence is detected	The membrane may not have been washed to remove the previous chemiluminescent substrate before stripping.	Wash the membrane first, then repeat the stripping procedure.
Antibody removal is incomplete after the wash	The post-strip PBS-T/TBS-T wash may be too short.	Wash for at least 30 minutes at room temperature; extending the wash to 1 hour may improve removal depending on the antibody.
Reprobing begins without confirming stripping	Residual antibodies may still be present.	Expose the stripped membrane to film or an imaging system to verify sufficient removal before the next immunoblot.
Wrong membrane type is being used	The datasheet specifies nitrocellulose and PVDF membranes.	Use the solution on the supported membrane types or separately validate other membrane materials.
Stripping performance varies between runs	The beta-mercaptoethanol may not be added freshly or incubation conditions may vary.	Prepare the reducing stripping solution immediately before use and standardize time, temperature, and volume.
Second immunoblot starts from the wrong step	The membrane may have been taken back through unnecessary upstream steps.	After successful stripping and washing, start the next immunoblot from the blocking step.
Insufficient solution contacts the membrane	Less than the recommended volume may leave parts of the membrane untreated.	Use 5-10 mL stripping solution per membrane and ensure full contact during the 55°C incubation.

Still having trouble? Recheck the pre-wash, 8 μ L/mL beta-mercaptoethanol, 5-10 mL volume, 1 hour at 55°C, at least 30 minutes of PBS-T/TBS-T washing, and verification imaging before reprobing.