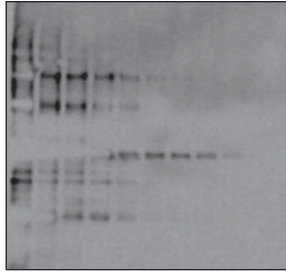


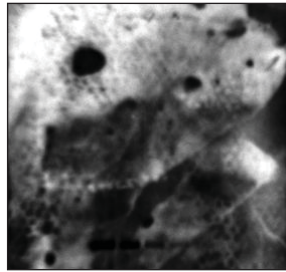
Five *Fast Fixes* for Chemiluminescent Western Blot Disasters

Screaming (Hot) Blots



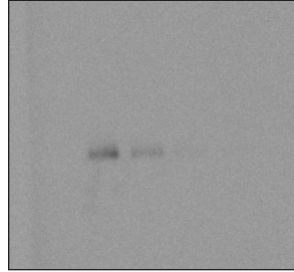
- Bands have white centers or “halos”.
- Bands swell outside their lanes.
- Exposures are hard to capture.

Unruly Blots



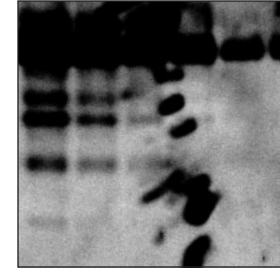
- Background completely covers blot.
- Background obscures signal.

Bashful Blots



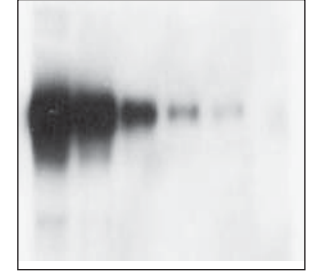
- Bands are weak or non-existent.
- Signals take a while to appear.

Non-specific Blots



- Multiple bands within a given lane when only one is expected
- Sample appears “stuck” in the stacking gel.

Temperamental Blots



- All other variables have been checked and problems still exist.

Possible Problem

- | | | | | |
|--|--|---|--|---|
| <ul style="list-style-type: none"> • Development/exposure time is too long. • Secondary antibody concentration is too high. • Sub-optimal substrate selection | <ul style="list-style-type: none"> • Cross-reactivity between the antibody and blocking solution • Washes need to be improved. • Too much substrate left on blot. | <ul style="list-style-type: none"> • Conjugate concentration is too low. • Substrate is not sensitive enough. • Blocking solution is interfering with signal generation. | <ul style="list-style-type: none"> • Overloading of target antigen • Non-specific reaction of primary or secondary antibody • Inadequate blocking | <ul style="list-style-type: none"> • Membrane choice may affect results. • Other reagents may be necessary to optimize substrate performance. |
|--|--|---|--|---|

Possible Solution

- | | | | | |
|---|---|--|--|---|
| <ul style="list-style-type: none"> • Reduce exposure time. • Reduce concentration of secondary antibody. • Choose less sensitive substrate (ex. LumiGLO®). | <ul style="list-style-type: none"> • Reduce cross-reactivity by trying other blocking solutions. • Increase number of washes or increase soak times during wash procedure. • Remove excess substrate from membrane before imaging. | <ul style="list-style-type: none"> • Increase conjugate/secondary antibody concentration. • Select a more sensitive substrate (ex., LumiGLO Reserve™, LumiGLO Ultra™) • Utilize a blocker with better signal:noise (ex., SigmaLOCK™). | <ul style="list-style-type: none"> • Reduce concentration of target antigen. • Check specificity of primary and secondary antibody. • Increase concentration of blocking reagent; increase blocking incubation time | <ul style="list-style-type: none"> • Try a complete optimized kit. • Switch membranes (nitrocellulose to PVDF or PVDF to nitrocellulose). • Check manufacturer's instructions for recommendations concerning membrane or other reagents. |
|---|---|--|--|---|

See reverse side for KPL products that can help resolve your blotting disasters!

Western blot kits - optimized for ultimate signal



SignalLOCK™ HRP ChemiWestern Kit (Imager)

Catalog# 54-54-00

- Optimized for femtogram-level detection using imager.
- HRP substrate, blocker, and wash

SignalLOCK™ HRP ChemiWestern Kit (Film)

Catalog# 54-53-00

- Optimized for femtogram-level detection on film.
- HRP substrate, blocker, and wash

SignalLOCK™ AP ChemiWestern Kit (Film/Imager)

Catalog# 54-56-00

- Optimized for femtogram-level detection.
- AP substrate, blocker, and wash.

Protein Detector LumiGLO® Western Blot Kit

Catalog# 54-12-50

- Fully optimized kit provides picogram detection.
- HRP substrate, blocker, wash, secondary antibody

Blockers - effective as antibody diluents and membrane blockers



SignalLOCK™ - Catalog# 50-58-00

- Non-animal origin, protein free
- Ultimate blocker to improve signal:noise

5X Detector™ Block - Catalog# 71-83-00

- Non-mammalian protein formulation helps minimize cross-reactivity.
- Premier blocker improves signal:noise.

Milk Diluent/Block - Catalog# 50-82-01

- Easily dilutable concentrate, no powders
- Can be used as both block and diluent.

10% BSA Diluent/Block - Catalog# 50-61-00

- Concentrated solution may be diluted for optimal use.
- Can be used as both block and diluent.

Washes - reduce nonspecific binding to the solid phase



20X Wash Solution- Catalog# 50-63-00

- Excellent all-purpose wash solution.
- Unique formulation (imidazole-buffered saline with Tween 20).
- Can be used in most Western protocols.

10X Phosphate-buffered Saline with Tween - Catalog# 51-14-01

- Ideal for use as a wash buffer in immunoassay applications.

10X Tris-buffered Saline w/Tween - Catalog# 51-18-01

- General wash buffer in immunoassay applications.

Chemiluminescent Substrates - improve detection



LumiGLO® Ultra Chemiluminescent Substrate Catalog # 54-51-00

- Optimized for femtogram-level detection using imager.

LumiGLO Reserve™ Chemiluminescent Substrate - Catalog# 54-71-00

- “Super Sensitive” chemiluminescent substrate.
- Provides excellent linearity and sensitivity in the femtogram range; optimized for film.

LumiGLO® Chemiluminescent Substrate Catalog# 54-61-00

- “Standard Use” chemiluminescent substrate.
- Provides excellent linearity and sensitivity in the low picogram range.

For help call the experts at 800.638.3167 or email techserv@kpl.com. See our comprehensive offering of blotting reagents by visiting www.kpl.com/westernblot.



www.seracare.com
800.676.1881
508.244.6400



www.kpl.com
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