USER MANUAL

One-Bottle Chemiluminescent Substrates

Western Lightning™ ONE

PICO

FEMTO

FEMTO ULTRA

Storage: Room temperature (18 - 25° C)

Version: 1.1 Date: December 2023

For research use only. Not for use in diagnostic procedures.

Western Lightning™ ONE

One-Bottle Chemiluminescence Substrates
for Western Blotting

Western Lightning ONE	Signal Intensity	Signal Duration	Protein Abundance	Available Sizes	Catalog Numbers
Pico	Medium	Short	High	25 mL	NEL130001EA
				250 mL	NEL131001EA
				500 mL	NEL132001EA
Femto	High	Medium	Medium	25 mL	NEL140001EA
				250 mL	NEL141001EA
				500 mL	NEL142001EA
Femto Ultra	Ultra-High	Short	Ultra-Low	25 mL	NEL150001EA
				125 mL	NEL151001EA
				250 mL	NEL152001EA

Western Lightning ONE is a 1-bottle substrate that can be stored at room termperature (18 - 25°C).

Western Lightning ONE is Revvity's premier line of premixed chemiluminescent substrates for Western blotting with protein detection sensitivity from picogram to low femtogram levels. Based on proprietary technology, these premixed solutions enable an increased experimental consistency by avoiding pipetting errors and possible contamination.

Important Information

- All Western Lightning ONE reagents have been specifically formulated and quality-controlled to detect proteins in Western blots. FOR RESEARCH USE ONLY.
- Proper blocking and washing of membranes is critical for optimum results. The recommended blocking and washing conditions should be tried first and adjusted as necessary for a particular application. For suggested blocking and washing reagents, see "Table 1: Reagents Needed".
- To achieve the maximum signal to noise ratio, the primary and secondary antibodies should be optimized in a titration experiment (See "Table 2: Suggested Antibody Dilutions")
- Phosphate buffers should not be used when phospho- proteins are being detected.
- Do not use kit components beyond the expiration date. This date is printed on the kit label.
- All Western Lightning ONE substrates can be stored at room temperature and are ready to use.

PROCEDURE SUMMARY

I. Membrane Preparation

Separate proteins by electrophoresis and transfer to PVDF or Protran™ nitrocellulose membrane.

II. Membrane Blocking

1. Block non-specific binding sites by incubating the membrane in 5% non-fat dry milk in TBST or other blocking reagent as appropriate for at least one hour or overnight at 4°C with gentle agitation.
2. Wash the membrane three times for 5 minutes with TBST.

III. Antibody Incubation

1. Dilute the primary antibody in 1% BSA/PBST or 1% BSA/TBST with the suggested primary antibody dilution (see table below) and incubate with the membrane for at least one hour or overnight at 4°C with gentle agitation. Make sure the entire surface of the blot is covered.
2. Wash the membrane with PBST or TBST once for 15 minutes, and then four times for 5 minutes each.
3. Dilute the HRP-labeled second antibody in 1% BSA/PBST or 1% BSA/TBST and incubate with the membrane for at least one hour or overnight at 4°C with gentle agitation.
4. Wash the membrane with PBST or TBST once for 15 minutes and then four times for 5 minutes each. The membrane may be left in buffer overnight at 4°C with gentle agitation.

IMPORTANT: To obtain the best outcomes, it is crucial to optimize both the primary and secondary antibody dilutions. This will result in high signal and low background. Optimal antibody dilutions may vary between different applications and depend on quality and affinity for the target protein.

SUGGESTED ANTIBODY DILUTIONS

Product	Proposed Antibody Concentrations
Western Lightning ONE Pico	**Primary Ab** 1:100 - 1:5,000 **Secondary Ab** 1:1,000 - 1:15,000
Western Lightning ONE Femto	**Primary Ab** 1:1000 - 1:15,000 **Secondary Ab** 1:25,000 - 1:150,000
Western Lightning ONE Femto Ultra	**Primary Ab** 1:5000 - 1:100,000 **Secondary Ab** 1:100,000 - 1:500,000

IV. Chemiluminescence Detection

All Western Lightning ONE substrates are premixed and can be used straight from the bottle.

1. Incubate the membrane in Western Lightning ONE substrate for one minute with gentle agitation.
2. Remove excess substrate by draining or blotting, and place the membrane in a plastic sheet protector.
3. Detect proteins according to desired method below:
   a. For detection on film: Expose to BioMax Light or X-OMAT Blue Autoradiography Film for 30 seconds. Develop the film and if necessary, use the result to determine an optimum exposure.
   b. For detection on imaging devices: Use optimum settings for chemiluminescence with luminol as recommended by the manufacturer.

V. Stripping and reprobing *(Optional)*

A nitrocellulose or PVDF membrane blot can be stripped and reprobed using this protocol up to four times.

1. After the film or CCD exposure, wash the membrane for 4 X 5 minutes in PBST or TBST.
2. Incubate the membrane for 30 minutes at 50°C in stripping buffer.
3. Wash the membrane 6 times for 5 minutes each in PBST or TBST.
4. Incubate the membrane for 1 minute in the previously used Western Lightning ONE substrate. Expose to film or CCD for 1 minute to 1 hour to make sure that the original signal is removed.
5. Wash the membrane again 4 times for 5 minutes each in PBST or TBST.
6. The membrane is now ready for reuse. Start at Membrane Blocking II in this Procedure.

REAGENT PREPARATION

<table>
<tr>
<td>10X Phosphate Buffered Saline (10X PBS)
For 1 liter: $NaH_2PO_4.H_2O$ 2.03 g
Na_2HPO_4 11.49 g
NaCl 85 g
Adjust to pH to 7.3 to 7.5 with HCl.
Storage: Room Temperature.
Alternately, Dulbecco's Phosphate Buffered Saline without
calcium chloride or magnesium chloride (available from commercial sources). Do not use phosphate buffers when detecting phosphoproteins.</td>
<td>10X Tris Buffered Saline (10X TBS)
For 1 liter: Tris base 24.23 g
NaCl 87 g

Adjust to pH to 7.3 to 7.5 with HCl.
Storage: Room Temperature.</td>
</tr>
<tr>
<td>10X PBS-TWEEN® 20 (10X PBST)
For 1 liter: 10X PBS 995 ml
TWEEN® 20 5 ml
A preservative such as thimerosal (1 g/L) may be added to prolong the life of the reagent. Do not use sodium azide because it inhibits HRP activity.
Storage: Room Temperature.</td>
<td>10X TBS-TWEEN® 20 (10X TBST)
For 1 liter: 10X TBS 995 ml
TWEEN® 20 5 ml
A preservative such as thimerosal (1 g/L) may be added to prolong the life of the reagent. Do not use sodium azide because it inhibits HRP activity.
Storage: Room Temperature.</td>
</tr>
<tr>
<td>1X PBST
For 1 liter: 10X PBS-T 100 ml
dH_2O 900 ml
Storage: Room Temperature</td>
<td>1X TBST
For 1 liter: 10X TBS-T 100 ml
dH_2O 900 ml
Storage: Room Temperature</td>
</tr>
<tr>
<td colspan="2">Membrane Blocking Buffer (5% Non-Fat Dry Milk)
For 100 ml: Carnation™ Instant Non-Fat Dry Milk 5 g
1X PBST or 1X TBST 100 ml
If additional blocking capability is desired, this reagent may be supplemented with normal serum of the same type as the antibody. Casein or BSA may be substituted for the non-fat dry milk. This reagent should be made up fresh for every use.</td>
</tr>
<tr>
<td colspan="2">Antibody Diluent (1% BSA)
For 1 liter: 10X PBST or TBST 100 ml
H_2O 800 ml
BSA 10 g
Adjust the pH to 7.4, add H_2O to 1 liter, and filter through a 0.22 µm membrane.
Storage: 4°C</td>
</tr>
<tr>
<td colspan="2">Stripping Buffer
62.5 mM Tris-HCl pH 6.8
2% SDS
100 mM 2-mercaptoethanol</td>
</tr>
</table>

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Remedy
No signal / weak signal	Poor transfer of proteins	Check gel. Use Colored MW Markers. Use correct pore size membrane for proteins >20 kD use a 0.45 µm membrane <20 kD use a 0.22 µm membrane
	Detergents, SDS, exhibit poor binding of low MW proteins	Remove SDS whenever possible
	Membrane preparation inadequate	Check proper membrane hydration Alcohol-Water-Buffer
	Primary or secondary antibody concentration too low, too high or inactive	Titrate antibody conjugates for optimum concentration or make up fresh
	Wrong blocking reagent	Test Blocking reagents with proteins for non-affinity
	Azide inhibiting HRP activity	Use only azide-free reagents
	Chemiluminescence reagent improperly prepared	Add HRP conjugate to reagent and look for visible light in a darkroom
	Precipitation of components in luminol or oxidizing solutions because of freezing	Mix moderately to ensure that all components are in solution
Excess signal / Non-specific Binding	Antigen or antibody excess	Adjust concentrations by optimization experiments
	Primary antibody is not specific for the protein of interest	Use monospecific or antigen affinity purified antibodies.
	Non-specific binding of secondary antibody	Confirm the secondary is specific by omitting the primary and running a secondary only blot. If bands develop, choose an alternative secondary antibody.
	Aggregation of analyte	Increase amount of reducing agent to ensure complete reducing of disulfide bonds.
	SDS interference	Presence of SDS may result in development of unspecific bands caused by antibodies binding to the charged SDS molecules associated with the proteins. Wash the membrane thoroughly with water after transfer.
High background	Antigen or antibody excess	Adjust concentrations by optimization experiments
	Cross Reactivity of Blocking Reagent & Antibody	Test blocking buffers or use Tween-20 in Wash Buffer
	Overexposure to film / exposure time imaging device set too long	Shorter film exposure or let signal decay for 10-15 minutes and repeat exposure. For the imaging device, use a shorter exposure time.
	Membrane dried out during incubation	Use enough reagent to keep membrane wet
	Insufficient washing	Increase the volume, length, and number of wash steps
	Poor quality antibodies	Use good quality affinity purified antibodies
	Membrane died out	Use enough reagent to keep membrane wet during incubation

 Troubshooting continued on next page ➡

Problem	Possible Cause	Remedy
White bands or "ghost" bands	Blank bands on film caused by depletion of chemiluminescence substrate at sites of excess antigen and/or antibody	Reduce concentration of the secondary HRP labeled antibody
"Blotchy" blot	Fingerprints, metal forceps, gloves	Use powder free gloves and avoid touching or gloves folding the membranes
Speckled background	Blocking Reagent Secondary HRP conjugated Ab	Filter using 0.45 µm aqueous filter Spin for 10-20 seconds, use supernatant

RELATED PRODUCTS

Category	Part Number	Products	Unit Size
Membranes	NBA083C001EA	Protran™ Nitrocellulose (0.2 µm pore size)	1 (30 cm x 3 m) roll
	NBA085C001EA	Protran Nitrocellulose (0.45 µm pore size)	1 (30 cm x 3 m) roll
Secondary Reagents	NEF812001EA	Anti-rabbit IgG (goat) HRP	1 mg, 1 mg/ml
	NEF822001EA	Anti-mouse IgG (goat) HRP	1 mg, 1 mg/ml
	NEF802001EA	Anti-human IgG (goat) HRP	1 mg, 1 mg/ml
	NEL750001EA	Streptavidin-HRP	2 vials, 0.25 mL/vial

*Products available from Biolegend, a Revvity company

Category	Part Number	Products*	Unit Size
Protein Ladders	773301	Prime-Step Prestained Broad Range Protein Ladder	50 µL
	773302	Prime-Step Prestained Broad Range Protein Ladder	500 µL
Secondary Reagents	410604	Direct-Blot HRP anti-human IgG1 Fc Antibody	25 µL
	410603	Direct-Blot HRP anti-human IgG1 Fc Antibody	100 µL
	410601	Purified anti-human IgG1 FC Anibody	100 µg
	408804	Biotin Anti-mouse IgE[8] Antibody	500 µg
	410406	HRP anti-rabbit IgG Antibody	100 µL
	410404	Purified anti-rabbit IgG Antibody	100 µg
	405306	HRP Goat anti-mouse IgG (minimal x-reactivity) Antibody	500 µL
	405301	Purified Goat anti-mouse IgG (minimal x-reactivity) Antibody	500 µg
	405428	Biotin Goat anti-rat IgG (minimal x-reactivity) Antibody	100 µg
	405501	Biotin Goat anti-hamster (Armenian) IgG Antibody	500 µg
	405601	Biotin Goat anti-hamster (syrian) IgG Antibody	500 µg
	406401	HRP Donkey anti-rabbit IgG (minimal x-reactivity) Antibody	500 µL
Western-Ready Buffers	426307	Western-Ready MES SDS-PAGE Running Buffer (10X)	1 L
	426311	Western-Ready Proein Sample Loading Buffer (5X)	20 mL
	426305	Western-Ready Rapic Protein Extaction Buffer	100 mL
	426309	Western-Ready TBS Tween-20 Buffer (20X)	1 L
	426313	Western-Ready Trasnfer Buffer (10X)	1 L

For further information on blotting reagents, membranes, and related products, please contact your local Revvity representative or visit : https://www.revvity.com

For Technical Support, please contact : LS.ReagentsTechSupport@revvity.com

References

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Heindl, D. and Josel, H.P. (1997) Non-radioactive Analysis of Biomolecules, 258-261. Springer, Berlin.

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