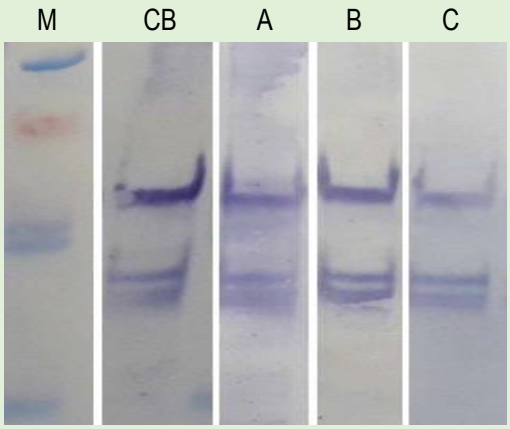
ChonBlock™ Western Blot Buffers

Traditional western blot buffers containing BSA, Casein, and NGS provide an economical method for increasing the signal-to-noise ratio in western blot analysis. Many proprietary western blot buffers are more costly than these traditional buffers but provide substantial blocking effects. ChonBlock™ is a new western blot buffer that proves to be just as capable as other proprietary buffers and at a cost-effective price. For more information about this product, please contact Chondrex, Inc. at support@chondrex.com.

1. Comparing the Effectiveness of ChonBlock™ to Competitor Western Blot Buffers

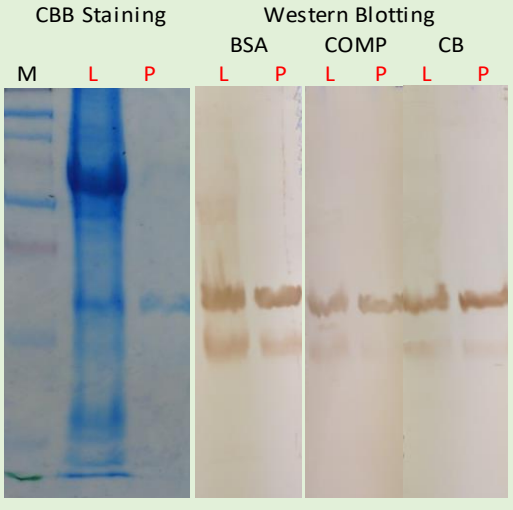


M: Molecular Marker
CB: ChonBlock™
A: Competitor's Buffer A
B: Competitor's Buffer B
C: Competitor's Buffer C
1: HMGB1
2: Degraded HMGB1

Reagents:
- Sample: Cell lysate containing purified HMGB1
- Primary antibody: anti-HMGB1 mAb (Cat # 7032, 1 µg/ml)
- Secondary antibody: goat anti-mouse IgG HRP (1:5000 dilution)

	BSA	Competitor	ChonBlock™
Signal	High	Low	High
BG Noise	Significant	None	None
Specificity	Low	High	High
Price	$	$$$	$$

2. Comparing the Effectiveness of ChonBlock™ to Common Western Blot Buffers



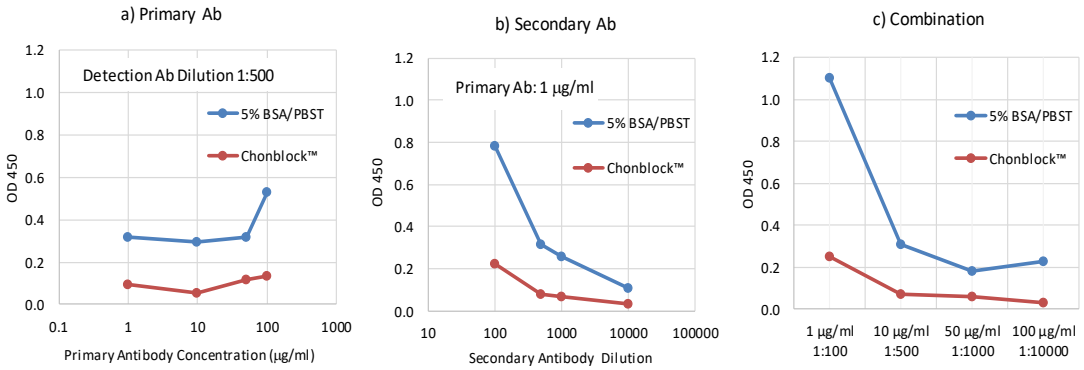
L: SP2/O Cell Lysate
P: Purified HMGB1 (Cat # 9050)
M: Molecular Marker
BSA: Bovine Serum Albumin-Tween
CBB: Coomassie Brilliant Blue
CB: ChonBlock™
COMP: Competitor's Buffer
1: HMGB1
2: Degraded HMGB1

A western blot was run using a 15% poly-acrylamide gel under reducing conditions to compare three different buffer systems. Mouse anti-HMGB1 monoclonal antibody (Cat # 7032) was used as the primary antibody at 1 µg/ml, and goat anti-mouse IgG antibody conjugated with HRP was used as the secondary antibody at a 1/4000 dilution. A non-specific smear signal was observed in the BSA-Tween buffer due to the lack of blocking effect. The competitor's buffer prevented non-specific smearing but showed a weak signal with high background color. In contrast, a distinct HMGB1 band with low background was observed using ChonBlock™.

	ChonBlock™	Competitor A	Competitor B	Competitor C
Signal	High	Moderate	Moderate	Low
Background Noise	Low	Low	None	None

ChonBlock™ Western Blot Buffers

The Blocking Effectiveness of ChonBlock™ and 5% BSA-0.05% Tween 20 on BG Noise Reactions



The background (BG) noise reaction in western blotting is caused by non-specific hydrophobic binding of antibodies to the nitrocellulose membrane. To determine the blocking ability of ChonBlock™ as compared to a conventional blocking buffer, small nitrocellulose membrane pieces were blocked with either ChonBlock™ or BSA-Tween buffer and reacted with primary and secondary antibodies in an ELISA plate. Color was developed using TMB (for ELISA) and OD values in each well were quantitatively obtained using a plate reader. As seen in the above graphs, the BG noise OD values depend on antibody concentration (a & b) and consequently are enhanced by their combination (c). ChonBlock™ prevents BG noise reaction more effectively than BSA.

Catalog #	Description
9069P (PBS) 9069T (TBS)	ChonBlock™ WB Buffer-1 for Blocking/Primary Antibody Dilution (x100 concentrate), 10 ml
90691P (PBS) 90691T (TBS)	ChonBlock™ WB Buffer-2 for Goat Secondary Antibody Dilution (x100 concentrate), 10 ml
90692P (PBS) 90692T (TBS)	ChonBlock™ WB Buffer-2 for Rabbit Secondary Antibody Dilution (x100 concentrate), 10 ml

Western Blot Protocol: Choose PBS or TBS-based Chonblock™ based on your study needs.

1. Prepare a 1X ChonBlock™ WB Buffer-1 solution by diluting with PBS or TBS.
2. Block the membrane with 1X ChonBlock™ WB Buffer-1 at room temperature for 1 hour.
3. Prepare the primary antibody solution by diluting with 1X ChonBlock™ WB Buffer-1.
   NOTE: Optimize the primary antibody concentration before use.
4. Incubate the membrane in the primary antibody solution at 4°C overnight.
5. Wash the membrane with PBS-Tween or TBS-Tween for 5 minutes, 3 times.
6. Prepare 1X ChonBlock™ WB Buffer-2 by diluting with PBS or TBS.
7. Prepare the HRP-conjugated secondary antibody solution by diluting with 1X ChonBlock™ WB Buffer-2.
   NOTE 1: Choose ChonBlock™ WB Buffer-2 for goat or rabbit depending on the secondary antibody's origin.
   NOTE 2: Use secondary antibodies pre-adsorbed against the same species as the sample.
   NOTE 3: Optimize the secondary antibody concentration before use.
8. Incubate the membrane in the HRP-conjugated secondary antibody solution at room temperature for 1 hour.
9. Wash the membrane with PBS-Tween or TBS-Tween for 5 minutes, 3 times.
10. Develop bands using desired chromogen, 3,3'-Diaminobenzidine (DAB) or 3,3', 5,5;-tetramethylbenzidine (TMB), for 5-30 minutes
11. Rinse the membrane with distilled water and let dry.