3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Chemichrome® Western Control
For Western blotting systems using anti-mouse secondary antibodies

Product Number **C2242**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Chemichrome™ Western Control is a positive control for use in Western blotting. It is designed for qualitative molecular weight determinations in Laemmli SDS-PAGE systems[1] and use as a visual confirmation of Western blot transfer efficiency.

Chemichrome Western Control contains eight proteins that have been chemically reduced, alkylated, and conjugated to brilliantly colored dyes. These eight proteins can be readily visualized in a gel or on a membrane after transfer. Chemically reduced and alkylated mouse IgG has also been added, so that Chemichrome Western Control can be used as a positive control for anti-mouse secondary antibody conjugates in either colorimetric or chemiluminescent systems. The heavy chain of the IgG (~50 kDa) is usually visualized after Western blotting. The light chain of IgG (25 kDa) may be visualized if a secondary antibody specific to the Fab region of the primary antibody is used.

Chemichrome Western Control is supplied as a ready-to-use solution that resists freezing.

- No need for the chemical reduction of the markers before loading the gel.
- No boiling is required.
- No freeze/thaw cycles means diminished degradation and longer shelf life.
- Storage at –20 °C conserves valuable –70 °C freezer space.

The product is supplied as a ready-to-use solution. Simply allow Chemichrome Western Control to warm to room temperature before use and then load onto a gel. Chemichrome Western Control transfers cleanly to PVDF or nitrocellulose membranes using Towbin's[2] or CAPS buffer, respectively.

Each vial of Chemichrome Western Control contains 200 µl of solution.

Reagents and Equipment Required But Not Provided

- SDS-PAGE gels, running buffer, and gel unit or apparatus
- Nitrocellulose (Product Code N5891) or PVDF (Product Code P4188) membrane
- Blotting paper (Product Code P7796)
- Western transfer buffer (Product Code T4904)
- Western blotting apparatus
- Blocking Solution (see Related Products)
- Methanol (Product Code M1775)
- Primary mouse antibody
- Anti-mouse secondary antibody
- For colorimetric detection, TMB substrate (Product Code T0565) for horseradish peroxidase (HRP) detection, BCIP/NBT substrate (Product Code B6404) for alkaline phosphatase (AP) detection, or a suitable chemiluminescent substrate for HRP or AP detection should be used.
- Tris buffered saline with TWEEN® 20 (TBST, Product Code T9039) or phosphate buffered saline with TWEEN 20 (PBST, Product Code P3563).

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Note: It is **not** recommended that Chemichrome Western Control be used as standards for quantitative molecular weight determinations, but only as a qualitative tool. For quantitative molecular weight determinations use the following:
SigmaMarker, Low Range, 6.5-66 kDa,
(Product Code M3913)
SigmaMarker, High Range, 36-205 kDa,
(Product Code S8320)
SigmaMarker, Wide Range, 6.5-205 kDa,
(Product Code S8445)

Storage/Stability
It is recommended to store the product at –20 °C. Chemichrome Western Control is stable for at least one year as supplied.

Procedure
Load Amount
Different substrates and different secondary antibodies in conjunction with different blocking agents will cause variation in the detection of the target protein(s). As a result, different amounts of Chemichrome Western Control should be loaded onto the gel depending on the blocking agent, secondary antibody, and substrate used. High sensitivity substrates and blockers need less Chemichrome Western Control than do the less sensitive substrates and blockers. See Table 1 for the recommended loading volumes for different blockers and substrates. If a Western blot is performed and the Chemichrome Western Control signal is too strong, either load less Chemichrome Western Control or dilute the Chemichrome Western Control in Laemmli sample buffer (Product Code S3401) before loading onto the gel.

Table 1.
Recommended loading volumes of Chemichrome Western Control per well

Blocking Reagent Used	**Detection System**		
	ECL™ and ECL Plus™	SuperSignal® West Femto and SuperSignal® West Dura	TMB and BCIP/NBT
	µl of Chemichrome Western Control per well*		
Western Blocker Solution	5 µl	2.5 µl	5 µl
Non-fat Dry Milk Blocker	10 µl	5 µl	5 µl

*This recommendation is appropriate for a gel of 1 mm thickness and a well of 5 mm width.

Western Blotting Procedure
This procedure assumes that the optimal amounts of primary and secondary antibodies are known. This is a general guideline procedure; each antibody-antigen pair must be optimized for signal by the end user.

1. All steps below should be performed with slight agitation on a rocker or orbital shaker such that the membrane is freely floating. All incubations should be performed at room temperature.
2. Load the gel with Chemichrome Western Control and samples (see Table 1).
3. Run the gel and then transfer to either a PVDF or nitrocellulose membrane.
4. Wash the membrane for at least 2 minutes with high purity water.
5. Block the membrane in appropriate blocking agent for 30–60 minutes (see Related Products for suggestions).
6. Add the primary antibody to the blocking agent. The final concentration of the primary antibody in this solution can range from 1-20 µg/ml.
7. Incubate the membrane with the primary antibody solution for at least 30 minutes.
8. Wash with TBST (Product Code T9039) or PBST (Product Code P3563) for at least 1 minute.
9. Remove the TBST or PBST and add at least 10 ml of appropriate blocking agent to the membrane. Add the appropriate amount of secondary antibody recommended by the detection substrate used.
10. Incubate the membrane with the secondary antibody solution for 30–60 minutes.
11. Remove the blocking solution and wash the membrane 4 times for 5 minutes each with TBST or PBST.
12. Remove the membrane from the wash buffer and drain any excess liquid from the membrane. Keep the membrane damp; do not allow the membrane to dry out.
13. Place the membrane on a flat sheet of plastic wrap or on any clean plastic surface.
14. Use the detection substrate (colorimetric or chemiluminescent) compatible with the secondary antibody (HRP or AP) used (see Related Products for suggestions).

Results
Table 2.

Apparent Molecular Weights (kDa) of the Proteins in Chemichrome Western Control		
Band Color	4 → 20% Tris-Glycine	10 → 20% Tris-Tricine
Violet	220	210
Pink	100	90
Blue	60	65
Pink	45	40
Orange	30	30
Blue	20	20
Pink	12	13
Blue	8	8

Apparent Molecular Weights were determined by using SigmaMarker, Wide Range, 6.5-205 kDa, (Product Code S8445) as a standard. The molecular weight of the violet band, which is outside the range of the standard, is an approximation.

Figures 1A and 1B.
Chemichrome Western Control in SDS-PAGE Gel and PVDF membrane

5 µl 2.5 µl

Figure 1A

5 µl 2.5 µl

Figure 1B

A. 10-20% Tris-tricine gel was loaded with 5 and 2.5 µl of Chemichrome Western Control. The gel was run using standard conditions on an 8 x 8 cm, 1 mm thick, 12-well precast gel.
B. Chemichrome Western Control transferred to PVDF membrane from the Tris-tricine gel. Transfer was completed in 60 minutes at 70 volts with Towbin's buffer (Tris-Glycine in 20% methanol).[2]

Figures 2A and 2B.
Colorimetric and Chemiluminescent development

1 2 3

Figure 2A

1 2 3

Figure 2B

PVDF membranes showing markers transferred from a 10-20% Tris-Tricine gel. Lane 1 is 5 µl of Colorburst marker (Product Code C4105), lanes 2 and 3 are 5 µl and 2.5 µl respectively of Chemichrome Western Control. The membrane was blocked with TBS with 3% milk (Product Code T8793).

A. 1:10,000 dilution of anti-mouse HRP antibody (Product Code A9044) was used. The membrane was developed with TMB (Product Code T0565) substrate for 20 minutes then rinsed with water.
B. 1:300,000 dilution of anti-mouse HRP antibody (Product Code A9044) was used. The membrane was developed with chemiluminescent HRP substrate and exposed to film for 30 seconds.

Related Products

Product Name	Product Code
TBS	T6664
PBS	P3813
Western Blocker Solution	W0138
TBS with 3% milk	T8793
PBS with 3% milk	P2194
TBS with TWEEN 20	T9039
PBS with TWEEN 20	P3563
Anti-mouse HRP Ab	A9044
Anti-mouse AP Ab	A3562
TMB substrate	T0565
BCIP/NBT substrate	B6404

References

1. Laemmli, U.K., Nature, **227**, 680-685 (1970).
2. Towbin, H., et al., Proc. Natl. Acad. Sci. USA, **76**, 4350-4354 (1979).

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