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ProductInformation

Chemichrome Western Control
For systems using anti-mouse secondary antibodies

Product Code **C 4236**
Storage Temperature –20 °C

Product Description

Chemichrome Western Control is used as a positive control in Western blotting. The Western Control is designed for qualitative determination in Laemmli SDS-PAGE systems,[1] and for use as a visual check of Western transfer efficiency. 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.

Chemichrome Western Control is composed of 8 proteins which have been chemically reduced, alkylated and conjugated to brilliantly colored dyes, plus mouse IgG that has been chemically reduced and alkylated. The 8 dyed proteins can be readily visualized in a gel or on a membrane after transfer. The heavy chain of the mouse IgG (approximately 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 is used.

Chemichrome Western Control transfers cleanly to nitrocellulose or PVDF membranes using Towbin's[2] or CAPS buffer, respectively.

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 saves on precious –70 °C freezer space.

Components

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

Components Necessary but not Provided

- SDS-PAGE gels
- Running buffer (T 7777 or T 1165 depending on the type of gel used)
- Electrophoresis apparatus
- Nitrocellulose (N 5891) or PVDF (P 4188) membrane
- Blotting paper (P 7796)
- Western transfer buffer (T 4904)
- Western Transfer apparatus
- Primary mouse antibody
- Anti-mouse secondary antibody:
  A 9044 a horse radish peroxidase (HRP) conjugate or A 5324 an alkaline phosphatase (AP) conjugate is recommended.
- For colormetric detection, TMB substrate (T 0565) for HRP detection or BCIP/NBT substrate (B 6404) for AP detection or suitable chemiluminescent substrate for HRP or AP detection should be used.
- Tris buffered saline with Tween 20 (TBST, T 9039) or phosphate buffered saline with Tween 20 (PBST, P 3563).

Precautions and Disclaimer

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 M 3913)
SigmaMarker, High Range (36-205 kDa)
(Product Code M 3788)
SigmaMarker, Wide Range (6.5-205 kDa)
(Product Code M 4038)

Preparation Instructions
Product is supplied ready to use. Just remove from the freezer, warm to room temperature and then load onto gel.

Storage/Stability
Store at –20 °C. 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 your target protein(s). As a result, different concentrations of Chemichrome Western Control should be loaded on the gel depending on the blocking agent, secondary antibody and substrate used. High sensitivity substrates and blockers need less Chemichrome than less sensitive substrates and blockers. Following is a list of several blocker and substrates and the recommended loading amount.

Recommeded loading suggestions per well

	ECL™ and ECL+™	Supersignal West femto™ and Supersignal West Dura™	TMB and BCIP/NBT
Western blocker solution	5 µl	2.5 µl	5 µl
Nonfat dry milk blocker	10 µl	5 µl	5 µl

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 needs to be optimized for signal by the end user.

1. All steps below should be performed with slight agitation on a rocker or an 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 above for suggested loading of chemichrome).
3. Run gel then transfer to either PVDF or NC membrane.
4. Remove membrane from Western blotting apparatus and wash membrane for 1 minute in either TBST (T 9039) or PBST (P 3563). (Either a TBS or PBS system can be used for western blotting).
5. Block membrane in appropriate blocking agent for 30 minutes (see chart for suggestions).
6. Add primary antibody to the blocking agent. The final concentration of primary antibody in this solution can range from 5-20 µg/ml.
7. Incubate membrane with the primary antibody solution for at least 30 minutes
8. Wash with TBST or PBST for 1 minute.
9. Remove TBST or PBST and add at least 10 ml of appropriate blocking agent to the membrane. Add appropriate amount of secondary antibody recommended by the detection substrate used.
10. Incubate the membrane with the secondary antibody solution for 30 minutes.
11. Remove blocking solution and wash 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 let the membrane 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 chart for suggestions).

Results
Chemichrome Western Control in SDS-PAGE Gel and PVDF membrane

Figure 1A

Figure 1 B

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 8 x 8 cm, 1 mm thick, 12-well precast gels.

B) Chemichrome Western Control transferred to PVDF membrane from the above Tris-tricine gel. Transfer was completed in 60 mins at 70 volts with Towbin's buffer (Tris-Glycine in 20% methanol).[2]

Colormetric and Chemiluminescent development

1 2 3

Figure 3A

1 2 3

Figure 3B

PVDF membranes showing markers transferred from a 10-20% Tris-Tricine gel. Lane 1 is 5 µl of Colorburst marker (C 4105), lanes 2 and 3 are 5 µl and 2.5 µl respectively of Chemichrome Western Control. The membrane was blocked with TBS + 3% milk (T 8793).

A) 1:10,000 dilution of anti-mouse HRP antibody (A 9044) was used. The membrane was developed with TMB (T 0565) substrate for 20 min then rinsed with water.

B) 1:300,000 dilution of anti-mouse HRP antibody (A 9044) was used. The membrane was developed with chemiluminescent HRP substrate and exposed to film for 30 seconds.

Apparent Molecular Weights (kDa) of 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) as a standard. The molecular weight of the violet band, which is outside the range of the standard, is an approximation.

Related Products

Product Name	Package Size	Product Code
TBS	10 packets	T 6664
PBS	10 packets	P 3813
Western Blocker Solution	1000 ml	W 0138
TBS + 3% milk	10 packets	T 8793
PBS + 3% milk	10 packets	P 2194
TBS + Tween	10 packets	T 9039
PBS + Tween	10 packets	P 3563
Anti-mouse HRP Ab	2 ml	A 9044
Anti-mouse AP Ab	0.25, 0.5, 1ml	A 5324
TMB substrate	100 ml	T 0565
BCIP / NBT substrate	100 ml	B 6404

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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