Gel Negative Stain Kit for SDS-PAGE

Staining Kit for SDS-PAGE and Blotting

nacalai tesque
The quality for certainty.

Features

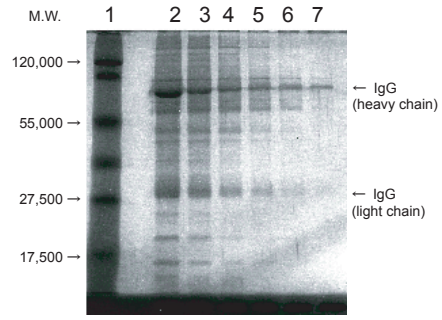
-The product temporarily detects proteins in SDS-PAGE before Western blotting.
-In this special technique, the background (the gel) turns milky white, while the protein bands remain transparent.
-The detection limit is as low as 1-10 ng, close to that of silver staining.
-The staining reaction takes about 20 minutes.
-Using the product for temporary staining before Western blotting only decreases detection efficiency by about 10 % in Western blotting.

Highly sensitive: detects proteins at nanogram level, similar to silver staining
Fast: staining takes only 20 minutes
Convenient: the same gel can be used for further Western blotting

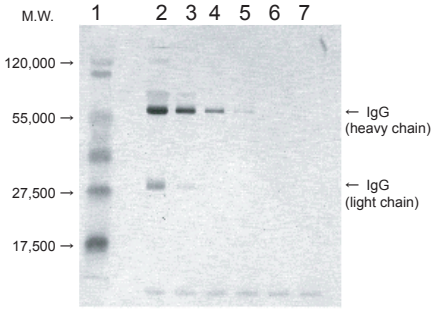
	Negative staining	CBB staining	Silver staining
Time	20 min	300 min	100 min
Sensitivity	1-10 ng	20 ng	1-10 ng
Number of steps	6 steps	2 steps	13 steps

Application

Western blotting after negative staining



Negative staining



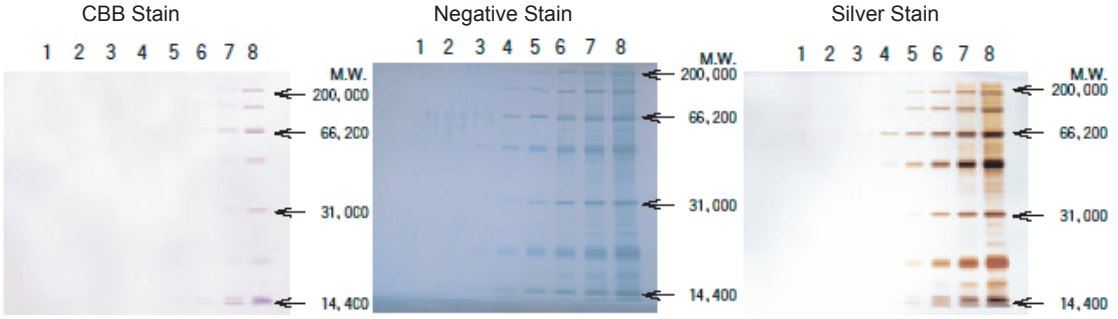
Western blotting after negative staining

< Condition >

Gel	: 12.5% polyacrylamide gel
Sample	: Human serum (reduced form)
Transfer	: 10V, 40min (constant voltage)
Membrane	: PVDF
Primary antibody	: Anti-Human IgG (goat)
Secondary antibody	: Anti-Goat IgG-AP conjugated (rabbit)
AP detection	: BCIP-NBT Solution Kit (Code No.: 03937-60)

Sample amount (2 µl/well) :
Lane 1 : Prestained Protein Markers (Code No.: 28941-75)
Lane 2 : 1/90 dilution
Lane 3 : 1/270 dilution
Lane 4 : 1/810 dilution
Lane 5 : 1/2430 dilution
Lane 6 : 1/7290 dilution
Lane 7 : 1/21870 dilution

Comparison



< Electrophoresis condition >
Gel : 12.5% Polyacrylamide gel
Sample: Protein Markers

Lane 1. 1.4 ng	Lane 2. 4.3 ng
Lane 3. 13 ng	Lane 4. 40 ng
Lane 5. 119 ng	Lane 6. 356 ng
Lane 7. 1100 ng	Lane 8. 3200 ng

Composition

Content	Components	PKG Size	Storage
Stock solution A (5X)	Sodium Lauryl Sulfate / Imidazole	1x 200 ml	RT
Stock solution B (5X)	Zinc sulfate	1x 200 ml	RT
Stock solution C (5X)	EDTA	1x 200 ml	RT

Preparation

(For staining a slab gel of 90 x 90 x 1 mm)
Use separate equipment (tray, pipette, etc...) for each solution (A, B and C) to prevent mixing. If the solutions mix, it may cause false result.

Solution A:
Put 40 ml deionized water into a clean 50-100 ml measuring cylinder, and mix in carefully 10 ml Stock solution A (5X) not raising foam.
Solution B:
Put 40 ml deionized water into a clean 50-100 ml measuring cylinder, and mix in carefully 10 ml Stock solution B (5X).
Solution C:
Put 40 ml deionized water into a clean 50-100 ml measuring cylinder, and mix in carefully 10 ml Stock solution C (5X).

Protocol

I. Staining
1. Wah the gel with 50 ml deionized water for 10 minutes.
2. Exchange the deionized water with 50 ml Solution A. Shake it for 5 minutes.
3. Remove the Solution A. Rinse the gel three times with 50 ml deionized water (shake for approx. 10 seconds). Note that rinsing too long may dilute the immigrated Solution A in the slab and cause weak staining result.
4. Pour 50 ml deionized water into a second plastic or stainless steel tray. Immerse the gel. Shake for 30-60 seconds till the bands appear. Note that the bands may become blurred, if immersed for more than 60 seconds.
5. Pour 50 ml deionized water into the tray used in step 3. Shake for 30 seconds, three times. Perform this rinsing process as soon as the bands appear.
6. The bands are easy to observe when the gel is placed on black plastic or stainless steel plate. Alternately, they can be clearly seen if the gel is held up to see-through light.

II.Destaining
1. Remove the deionized water from the tray used at step 5 in Staining. Shake the gel in 50 ml Solution C for 6 minutes. The gel will be destained and appear clear again.
2. Remove the Solution C. Rinse the gel with deionized water (shake for 30 seconds) three times.
3. After that, follow the general Western blotting protocol.

Reference

Fernandez-Patron, C., Hardy, E., Sosa, A., Seoane, J., and Castellanos., *(1995) Anal. Biochem., 224, 223-269*

Attention

-The staining time in step 4 in Staining protocol above changes according to the thickness of the gel. Expose (immerse) the gel to Solution B as long as the bands appear (usually between 30-60 seconds).
-Rinse the gel with deionized water immediately upon the appearance of the bands in step 4 in Staining. If the rinsing is not sufficient, the bands may become blurry gradually.
-Rinse the gel with deionized water throughly, after treatment with Solution C. If the rinsing is not sufficient, transfer efficiency at Western blotting may decrease.

Caution

-Handle the gel wearing protective gloves.
-The stained background will disappear in 1-2 days.
-Since Solution A contains SDS, white precipitation may occur after long cold storage. If that happened, immerse the bottle into warm water (approx. 30℃). After the precipitation dissolved, the product can be used.
-Solution A contains SDS and Solution B contains Zinc Sulfate. After use, discard Solution A as surfactant and discard Solution B as solution of zinc.

Expiration

One year from manufacturing. Expiration date is stated on the product label (Exp. yy / mm)

Ordering Information

Product name	Grade	Storage	Code No.	PKG Size
Gel Negative Stain Kit for SDS-PAGE	SP	RT	16660-41	1 kit

For research use only, not intended for diagnistic or drug use.

NACALAI TESQUE, INC.

Nijo Karasuma, Nakagyo-ku, Kyoto 604-0855, JAPAN
TEL: +81-75-251-1730
FAX: +81-75-251-1763
Web site: http://www.nacalai.com
E-mail: info.intl@nacalai.co.jp