Ver.1.00

Beacle, Inc.

Signal Booster

An immuno-reaction enhancing solution

Instruction

Beacle, Inc.
OKAYAMA JAPAN

---- Contents ----

Cautions

1. Research use only. Do not use for medical purpose.
2. Do not dilute or add other agents in Signal Booster solutions to get the best.
3. The color of Solution B is slightly yellowish as compared to Solution A, and not due to denaturizing.

(1) Introduction

Signal Booster is an enhancer of antigen-antibody reaction. In Western blotting and ELISA, researchers often experiences weak signal or high background. Signal Booster improves these problems by just using it as antibody diluents. Due to the principle of working mechanism, Signal Booster can be used in many assay systems that use antigen-antibody reaction.

How Signal Booster Works

Signal Booster contains a polymer which, by changing the physicochemical properties of antigen and antibody, enhances the mutual accessibility, and facilitate the specific reaction. The other ingredient, protein, reduces non-specific binding of antibody. Thus, Signal Booster enhances the antigen-antibody reaction while reduces background.

● Features of Signal Booster ●

1. High signal with low background

Signal Booster enhances the antigen-antibody reaction. Comparing with the method using detergent-containing buffer, several to over 10-fold stronger signal can be obtained while the background level is low. Thus, you can get much higher S/N ratio than usual method.

2. Effective for saving antibody usage and time of reaction time

Because higher signal can be obtained using Signal Booster, you can reduce the amount of antibody used and the time required for reactions.

3. Can be used for many reactions

Signal Booster can be used not only for Western blotting and ELISA, but also for other assay systems using antigen-antibody reactions. In addition, Signal Booster does not affect activities of HRP (horse radish peroxidase) or AP (alkaline phosphatase), and can be used for assay systems using these enzymes.

4. Easy to use

Signal Booster is formulated as to Ready to Use. Just exchange your dilution buffer of antibodies to the solutions of Signal Booster.

(2) Products

Signal Booster family has following products. This manual applies all these products.

<table>
<tr><th>Content \ Product #</th><th>BCL-101S</th><th>BCL-105</th><th>BCL-110</th><th>BCL-110
A, B</th><th>BCL-125</th><th>BCL-125
A, B</th></tr>
<tr><td>Solution A</td><td>20ml</td><td>50ml</td><td>100ml</td><td rowspan="2">A or B
100 ml</td><td>250ml</td><td rowspan="2">A or B
250 ml</td></tr>
<tr><td>Solution B</td><td>20ml</td><td>50ml</td><td>100ml</td><td>250ml</td></tr>
</table>

(3) How to use

- Signal Booster is consisted of Solution A and Solution B. Use Solution A for the dilution of the 1st antibody. Use Solution B for the dilution of the 2nd antibody. No change is required for the other assay protocol. For details see the later instructions.
- In some assay systems, only one antibody is used. For example some ELISA uses only one enzyme-conjugated antibody. In such case, try using Solution B for the antibody dilution. In some cases, however, Solution A gives better result. For details see the later instructions.
- Signal Booster has been successfully used in Western blotting, antibody sandwich ELISA with either the 1st or 2nd antibody-labeled type, antigen sandwich ELISA.

(4) Western Blotting (WB)

WB is a method to detect proteins by specific antibodies. Usually, proteins are separated by SDS-PAGE and transferred to membrane made by nitrocellulose or PVDF (polyvinylidene fluoride). The way to use Signal Booster in WB is described below.

1) SDS-PAGE and transfer of protein to PVDF membrane should be done by usual method.
2) Blocking and the washing should be done by usual method.
3) Dilute the 1st antibody with Solution A of Signal Booster. The dilution factor is influenced by many factors, such as antibody species, amount of antigen, etc. Though you can reduce antibody concentration by using Signal Booster, we recommend performing a pre-test to determine the best antibody concentration.
4) Dilute the 2st antibody with Solution B of Signal Booster. The best dilution factor is influenced by many factors, such as antibody species, amount of antigen, etc. Refer to the supplier's instruction to determine the best antibody concentration.
5) When using the enzyme-labeled 1st antibody and not using 2nd antibody, try using Solution B for dilution. In some cases, however, Solution A works better.
6) For visualization, many users use HRP-, or AP-labeled antibody. In both cases, please watch the strength of staining or luminescence and stop the reaction. Longer reaction gives you high background or appearance of extra band.

(5) ELISA

ELISA is a method to determine the amount of antigen or antibody in samples by using labeled antigen or antibody. The sandwich ELISA is most widely used, where antigen sample is applied on solid phase antibody and bound antigen is reacted by 1st antibody and visualized by labeled 2nd antibody. In some system, methods to use enzyme-labeled 1st antibody alone is also used. The way to use Signal Booster in these sandwich ELISAs are described below.

1) Antibody attachment (solid phase), blocking and the washing procedure should be done by usual method.
2) Dilute the antigen and 1st antibody with Solution A of Signal Booster. The dilution factor is influenced by many factors, such as antibody species, amount of antigen, and other factors, and is important to get good sensitivity. Please refer to the supplier's instruction to determine the best antibody concentration. Pour appropriate amount of these diluates into each well, mix and incubate for appropriate time. Alternatively, in some method, antibody diluent is added after the addition of antigen samples.
3) Dilute the 2st antibody with Solution B of Signal Booster. The best dilution factor is influenced by many factors, such as antibody species, amount of antigen, etc. Refer to the supplier's instruction to determine the best antibody concentration.
4) When using the enzyme-labeled 1st antibody and not using 2nd antibody, try using Solution B for dilution. In some cases, however, Solution A works better.
5) For detection, many users use HRP-, or AP-labeled antibody. In both cases, please perform pre-test to determine the best reaction time. Longer reaction gives you higher background.

(6) Trouble shooting

Trouble	Cause and resolutions
Western blotting	
Weak signal	1. Low antigen conc.: Use higher antigen conc.
	2. Low antibody conc.: Survey best antibody conc.
	3. Not enough transfer: Use higher current or longer transfer time.
	4. Blocking too strong: Do not use long time blocking.
	5. Too much transfer: When using nitrocellose, proteins pass though the membrane by strong transfer manipulation. Check the procedure or exchange membrane to PVDF.
Partial whitening (lumines.)	6. Too much antigen or antibody: Over signaling often suppress the luminescence and cause partial whitening of a band. Control the amount of antigen or antibody concentration.
Too many extra-bands	7. Too much higher antibody conc.: Higher antibody conc. often causes non-specific signaling. Control the antibody conc.
	8. Too much antigen: Higher antigen often causes non-specific signaling. Control the amount of antigen.
	9. Not enough blocking: Some antigen and antibody have preference of blocking agents, change the blocking agents or check the blocking conditions.
	10. Not enough washing: Increase the number and time of washing.
High background	11. High antibody conc. or too long incubation: Reduce the antibody conc. or shorten the incubation time.
ELISA	
Weak signal	1. Too low antigen or antibody conc.: Increase the concentrations.
Too strong signal	2. Too high antigen or antibody conc.: Check the antigen and antibody concentration by performing the titration.
	3. Too long incubation: shorten the incubation time.
High background	4. Too high antigen or antibody conc.: check antigen and antibody cocn.
	5. Not enough blocking: Some antigen and antibody preference of blocking agents, change the blocking agents or check the blocking conditions.
	6. Not enough washing or too much washing: check the number and time of washing.

(7) Contact Information

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【manufactured and distributed by】

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