Ver.1.01E

Beacle, Inc.

Signal Booster Immunostain

An immuno-reaction enhancing solution for immunostaining

Instruction

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Cautions

1. Research use only. Do not use for medical purpose.
2. Do not dilute or add other agents in Signal Booster Immunostain solutions to get the best result.
3. Solution F shows slightly yellowish color, and not due to denaturizing.

(1) Introduction

"Signal Booster Immunostain" is the enhancer of antigen-antibody reaction designed for immunostaining, and enhances specific signal antigen-antibody while reducing non-specific background staining. Thus, you can get high S/N ratio. Simply exchange the dilution buffer of antibody to Signal Booster Immunostain, then you can experience the observation with high S/N ratio. The product is produced based on the technology developed for Signal Booster which is an antigen-antibody reaction enhancer designed for Western Blotting and ELISA.

How Signal Booster Works ?

Signal Booster Immunostain contains polymers and proteins. One of these agents by changing the physicochemical properties of antigen and antibody, enhances the mutual accessibility, and facilitate the specific reaction. The other ingredients reduces non-specific binding of antibody. Thus, Signal Booster Immunostain enhances the antigen-antibody reaction while reduces background.

● Features of Signal Booster ●

1. Enhance specific signal while reducing background
   Signal Booster Immunostain enhances the specific antigen-antibody reaction, while reducing the non-specific binding . Thus, you can get much higher S/N ratio than usual method.
2. Can be used for many chromogenic reaction
   Signal Booster Immunostain does not affect activities of HRP (horse radish peroxidase) or ALP (alkaline phosphatase). Signal Booster Immunostain can be also used with signal enhancing systems such as ABC system.
3. Easy to use
   Signal Booster Immunostain is formulated as to Ready to Use. Just exchange your antibody dilution buffer to Signal Booster Immunostain.

(Caution: the signal enhancing effect of Signal Booster Immunostain greatly depends on the nature of antibody and antigen, and you may not always get good results)

(2) Products

Signal Booster Immunostain family has following products.

Product #	Composition	Content
BCL-IS	Set of Solutions F, M, and S	10 ml each
BCL-ISF	Solution F	20ml
BCL-ISM	Solution M	20 ml
BCL-ISS	Solution S	20 ml

For beginner users, we recommend to purchase BCL-IS as the initial try

(3) Characteristics of Compositions

Solution F: The solution is designed to maximize the background-reducing activity. Ideal for observing fine structures by using antibodies with high specificity and sensitivity.

Solution M: The solution shows characteristics of middle of F and S. Ideal for initial try.

Solution S: The solution is designed to maximize the specific signal enhancing activity. Ideal for observing strong signal by using antibodies with less specificity and sensitivity. This solution has less ability to reduce background.

(Caution: the above description shows general characteristics of the solutions, and the results you get may be different depending on the antibody and antigen you are interested in.)

(4) Method for paraffin sections

As an example, staining using ABC system is described below. If recommended procedure after secondary antibody reaction is indicated by ABC kit supplier, please follow the recommended procedure.

1) Deparaffinize sections by xylene, and hydrate by using series of ethanol solutions.
2) Wash sections with DW for over 5 min.
3) Denature the endogenous peroxidase by using 0.3 to 1.0 % H_2O_2 solution.
4) Rinse the section with DW for 5min and incubate for 5min in PBS two times.
5) Coat entire tissue sections with blocking solution, and incubate for 30 minutes at room temperature in a humid chamber. There is no limitation of the species of blocking agents.
6) Dilute primary antibody by one of three solutions of Signal Booster Immunostain to the concentration recommended by supplier.
7) After removal of blocking solution from sections, apply diluted primary antibody solution, and incubate for 1 hours at RT or overnight at 4℃ in humid chamber. Incubation time varies from antibody to antibody.
8) Wash sections with PBS for 5min three times.
9) Dilute biotinized secondary antibody by one of three solutions of Signal Booster Immunostain according to dilution factor recommended by supplier.
10) Apply diluted biotinized secondary antibody solution, and incubate for 30 min in humid chamber. Then, wash sections with PBS for 5min three times. (it is recommended to prepare avidin-biotin-peroxidase complex solution during this incubation time)
11) Apply the complex solution to the sections, and incubate for 30 min in humid chamber.
12) Wash sections with PBS for 5min three times, and incubate with chromatic substrate solutions.
13) Terminate reaction and mount the section using mounting medium. Observe by microscope.

(5) Method for frozen sections

For frozen sections, treat the section with the same manner as for paraffin sections except that treatment starts from 2) of above instructions. Be sure that the frozen sections are made from blocks of fixed samples and well dried. If sections are made from non-fixed samples, fix the sections with adequate fixative first and do the same procedure.

(6) Method for cultured cells

An example of cell staining using fluorescent-labeled secondary antibody is described.

1) Remove the culture medium and wash cultured cells once with PBS.
2) Fix cells for 30min with 4% paraformaldehyde in 0.1 M phosphate buffer at RT.
3) Wash cells with PBS for 5min three times.
4) Add blocking solution, and incubate for 30 min at RT.
5) Wash cells with PBS for 5min three times.
6) Dilute primary antibody by one of three solutions of Signal Booster Immunostain to the concentration recommended by supplier., and incubate for 1 hours at RT or overnight at 4℃ in humid chamber.
7) Wash cells with PBS for 5min three times.
8) Dilute fluorescent dye–labeled secondary antibody by one of three solutions of Signal Booster Immunostain to the concentration recommended by supplier, and incubate for 1 hours at RT.
9) Wash cells with PBS for 5min three times.
10) Observe the cells by fluorescent microscope.

(Caution: the addition of serum or protein into Signal Booster Immunostain alters the nature of the reagent, and affects the efficacy of the reagent)

(7) Trouble shooting

Trouble	Cause and resolutions
Weak signals	1. Not enough antigens. Antigens may be lost after section preparation. Examine the fixative or fixation procedure
	2. Low antibody concentration. Examine the antibody concentration.
	3. Antigen masked. Liberate antigen by antigen retrieval procedures or agents.
	4. Blocking too strong. Strong blocking may reduce the signal. Examine the blocking condition and the species blocking agent.
	5. Too much washing. Examine the time and number of washings and the composition of washing solution especially detergent concentration..
High background or appearance of nonspecific staining	6. Antibody concentration too high. Excess antibody can enhance non-specific signal. Examine the antibody concentration.
	7. Endogenous peroxidase activity remaining. When using peroxidase for chromogenic reaction, endogenous peroxidase enhances nonspecific staining. Use longer denaturing time or strengthen the denaturing condition.
	8. Not enough blocking. Some antigen and antibody have preference of blocking agents, change the blocking agents or check the blocking conditions.
	9. Not enough washing. Increase the number or time of washing.
	10. Too long incubation with antibody. Reduce the antibody concentration or shorten the incubation time.

(8) Related products

- Signal Booster:
  Antigen-antibody reaction enhancer for western blotting and ELISA.
- Easy-WESTERN:
  A kit for primary antibody detection system designed for western blotting. The key component is MAD reagents which is a HRP-conjugated FC region-binding particle. The kit enables us, without using ordinary secondary antibody, much advantages, such as highly sensitive detection, , multiple antigen detection, one-step detection and so on.

Explore much more by accessing our website; http://beacle.com

(9) Contact Information

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