COSMO BIO CO., LTD.
Inspiration for Life Science

Ver.1.00

IMMUNO SHOT

An immuno-reaction enhancing solution

Instruction

Product series

Cat number / Component	IS-012-20S	IS-012-100	IS-012-250	IS-001-100	IS-002-100	IS-001-250	IS-002-250
	20ml	100ml	250ml	100ml	100ml	250ml	250ml
Reagent 1	○	○	○	○		○	
Reagent 2	○	○	○		○		○

---- Contents ----

Cautions

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

(1) Introduction

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

How IMMUNO SHOT Works

IMMUNO SHOT 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, IMMUNO SHOT enhances the antigen-antibody reaction while reduces background.

Features of IMMUNO SHOT

1. High signal with low background

IMMUNO SHOT 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 IMMUNO SHOT, you can reduce the amount of antibody used and the time required for reactions.

3. Can be used for many reactions

IMMUNO SHOT can be used not only for Western blotting and ELISA, but also for other assay systems using antigen-antibody reactions. In addition, IMMUNO SHOT 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

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

(2) How to use

- IMMUNO SHOT is consisted of Reagent 1 and Reagent 2. Use Reagent 1 for the dilution of the 1st antibody. Use Reagent 2 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 Reagent 2 for the antibody dilution. In some cases, however, Reagent 1 gives better result. For details see the later instructions.
- IMMUNO SHOT has been successfully used in Western blotting, antibody sandwich ELISA with either the 1st or 2nd antibody-labeled type, antigen sandwich ELISA.

(3) 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 IMMUNO SHOT 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 Reagent 1 of IMMUNO SHOT. The dilution factor is influenced by many factors, such as antibody species, amount of antigen, etc. Though you can reduce antibody concentration by using IMMUNO SHOT, we recommend performing a pre-test to determine the best antibody concentration.
4) Dilute the 2nd antibody with Reagent 2 of IMMUNO SHOT. 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 Reagent 2 for dilution. In some cases, however, Reagent 1 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.

(4) 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 IMMUNO SHOT 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 Reagent 1 of IMMUNO SHOT. 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 dilutes 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 2nd antibody with Reagent 2 of IMMUNO SHOT. 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 Reagent 2 for dilution. In some cases, however, Reagent 1 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.

(5) 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.

COSMO BIO CO., LTD.
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TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
http://www.cosmobio.co.jp e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617 FAX : +81-3-5632-9618