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| Product | FastGene® Block & Go |
| Item Nr. | FG-CH05 |
| Description | Western blot blocking and enhancing solution |

## Storage conditions

Stable for up to 1 year at 25°C.

## Description

FastGene® Block & Go is a blocking solution for Western blot analysis. This buffer not only provides blocking and primary and secondary antibody hybridization in a single-step but also enhances the signal developed with HRP (horseradish peroxidase) or AP (alkaline phosphatase) substrates. Therefore, it serves as both blocker and enhancer in Western blot analysis. With the three-in-one step procedure, FastGene® Block & Go is a time and labor economic solution for the time consuming and laborious Western blot procedure.

## Required materials but not provided

- Primary antibody
- Secondary antibody conjugated with HRP.
- Wash buffer: PBST (phosphate buffered saline with Tween-20) or TBST (Tris buffered saline with Tween-20) buffer.
- ECL (Enhanced chemiluminescence) or colorimetric reagents
- Shaker: orbital or rocking shaker

## Reaction setup for single-step protocol

1. After Western blot transferring, immerse the PVDF or NC membrane in PBST/TBST buffer for 5 minutes.
2. Dilute the primary antibody and secondary antibody with proper amounts in 10 ml of FastGene® Block & Go each. For example, when the dilution factor for both the primary and the secondary antibodies is 1: 10,000, add 1 µl of the primary antibody to 10 ml of the FastGene® Block & Go (1st tube), followed by adding 1 µl of the secondary antibody to another 10 ml of the FastGene® Block & Go (2nd tube).
3. Thoroughly mix the antibody-FastGene® Block & Go solution inside each tube by inverting it back and forth several times.
4. Pour the primary antibody-FastGene® Block & Go solution into the prepared container first, followed by the addition of the secondary antibody-FastGene® Block & Go solution into the same container.

5. Incubate the membrane immediately in the antibody-FastGene® Block & Go solution at room temperature for 1 - 2 hours with gentle agitation. Please note that after mixing the primary and secondary antibodies, the membrane needs to be immediately immersed in the mixture within 10 minutes for obtaining the optimal performance.
6. Wash the membrane with PBST/TBST three times with shaking.
7. Drain excessive wash buffer and perform image development methods with ECL or colorimetric system immediately.s

## Reaction Setup for two-step protocol

1. After Western blot transferring, immerse the PVDF or NC membrane in PBST/TBST buffer for 5 minutes.
2. Dilute the primary antibody with proper amounts in 10 ml of FastGene® Block & Go. For example, when the dilution factor for the primary antibody is 1:10,000, add 1 µl of the primary antibody to 10 ml of the FastGene® Block & Go.
3. Thoroughly mix the antibody-FastGene® Block & Go solution by inverting it back and forth several times.
4. Incubate the membrane immediately in the antibody-FastGene® Block & Go solution at room temperature for 1 hour with gentle agitation.
5. Wash the membrane with PBST/TBST three times with shaking.
6. Dilute the secondary antibody with proper amounts in 10 ml of FastGene® Block & Go. For example, when the dilution factor for the secondary antibody is 1:10,000, add 1 µl of the secondary antibody to 10 ml of the FastGene® Block & Go.
7. Thoroughly mix the antibody-FastGene® Block & Go solution by inverting it back and forth several times.
8. Incubate the membrane immediately in the antibody-FastGene® Block & Go solution at room temperature for 1 hour with gentle agitation.
9. Wash the membrane with PBST/TBST three times with shaking.
10. Drain excessive wash buffer and perform image development methods with ECL or colorimetric system immediately.

## Important Notes

1. Please note that after mixing the primary and secondary antibodies, the membrane needs to be immediately immersed in the mixture within 10 minutes for obtaining the optimal performance.
2. The dilution for the secondary antibody should be at least 1:10,000, or more. A higher level of background noise will be observed as a result with a high concentration of secondary antibody.
3. Do not incubate the membrane in FastGene® Block & Go for over 4 hours to avoid high background. Overnight incubation is especially not recommended.
4. If the two-step protocol was applied, the primary antibodies mixed in FastGene Block&Go solution may be reused. Enhancing effect may trail off along with the increasing storage time or repetitiveness. Keep the mixed solution refrigerated. For critical experiment or strong signal, fresh preparation of antibody-FastGene® Block & Go solution is recommended.