For Research Use Only

Blotting Accelerator

Catalog Number: PR20039

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Description

This ready-to-use solution is specifically used for membrane blocking and antibody dilution in Western blot experiments and designed to significantly shorten the blotting workflow to around 40 minutes or less. It contains special organic compounds, which can significantly shorten the reaction time of experiments and thus improve experimental efficiency.
This product contains inert proteins of different molecular weight ranges, which can effectively reduce the background and protect the activity of the antibody. Antibody working solution diluted with this product can usually be stored at 4°C for more than 3 months.
This product contains pathology grade preservatives and is compatible with HRP, so it can dilute primary antibodies (including phosphorylated primary antibodies), HRP-labeled primary antibodies, or secondary antibodies. It is also possible to dilute fluorescently labeled primary and secondary antibodies.
Exclusion: This product contains BSA and cannot be used to dilute anti-BSA primary antibodies. This product is a semi-transparent colloid. Please do not use it if there are insoluble or lumpy aggregates.

Package

100 mL/500 mL

Storage

Store at 2-8°C for a year.

Usage

1. Electrophoresis and membrane transfer, according to the conventional method.
2. Blocking (optional). After transferring the membrane, place the blotting membrane in the reaction box, rinsing it with washing solution (TBST or PBST) for 3-5 seconds, then discard the washing solution. Add an appropriate amount of this product (completely covering the membrane) and place it on a horizontal shaker with slow speed, then block it for ~5 minutes at room temperature or 37°C.
Note: If this product is used as the diluent in the subsequent step, the blocking step can be omitted (i.e., discard the washing solution after rinsing and add the diluted primary antibody directly). If other diluent solutions are used in the subsequent step, or if the primary antibody reaction time is planned to be extended to more than 25 minutes, it is not recommended to omit the blocking step. A 5-minute blocking step with this product is sufficient to achieve the desired result, and an extension of the time will usually not make much difference. If you want to use this step as a stopping point for the experiment, you can proceed the blocking step at 4°C (30 minutes to 24 hours).
3. Primary antibody incubation.
3.1 Dilute the primary antibody in advance with this product. At the end of the blocking, discard the blocking solution and add an appropriate amount (completely covering the membrane) of primary antibody dilution. If there is excess primary antibody dilution, store it at 4°C.
Note: At the end of the blocking, the primary antibody dilution can be added directly without washing. If washing is required, it can be washed briefly once with a conventional washing solution.
3.2 Place the reaction box on a horizontal shaker, shake the primary antibody dilution quickly, then shake slowly for incubation at room temperature or 37°C for 10-25 minutes, which can be shortened to less than 5 minutes for some high-affinity antibodies.
3.3 At the end of primary antibody incubation, it is recommended to use PBST or TBST buffer for washing. Place the reaction box on a horizontal shaker and add an appropriate amount of washing solution to quickly wash twice (each time for 3-5 seconds). Then use the washing solution to wash three times (1 minute each time).
4. Incubation of secondary antibody (if the primary antibody is an HRP-labeled primary antibody, this step is not necessary; directly carry out step 5).
4.1 Dilute the secondary antibody with this product in advance. After the primary antibody dilution has been washed, add an appropriate amount (completely covering the membrane) of secondary antibody dilution. If there is excess secondary antibody dilution, store it at 4°C.
4.2 Place the reaction box on a horizontal shaker with fast shaking first, then incubate the secondary antibody for 8-15 minutes at room temperature or 37°C with slow shaking.
4.3 At the end of the secondary antibody incubation, it is recommended to use PBST or TBST buffer for washing. Place the reaction box on a horizontal shaker and add an appropriate amount of washing solution to wash rapidly twice (3-5 seconds each time). Then wash it with washing solution three more times (1 minute each time).
5. After washing, the substrate can be added for chemiluminescence imager detection or darkroom imaging operation.

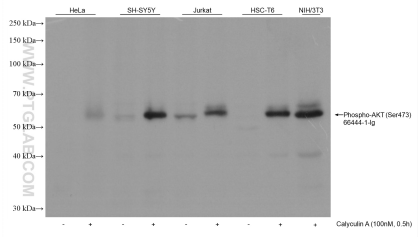
Notes

1. If you use this product for the first time, you may need to optimize the antibody dilution ratio. It is recommended to set the concentration of secondary antibody (generally 0.05-0.1 ug/mL) first, and optimize the concentration of primary antibody after setting the reaction time of primary antibody and secondary antibody.
2. If there are individual unsatisfactory results, you can adjust the concentration of the primary antibody, secondary antibody, or washing time, and optimize the imaging or exposure time appropriately.
3. The diluted primary antibody or secondary antibody can be stored at 4°C for more than 3 months. However, the length of storage time varies among different primary or secondary antibodies, so please make your judgment on the specific stability period.
4. This product contains the preservative ProClin 300. Please wear a lab coat and disposable gloves.
5. This product is only for professional laboratory scientific research use, not for medical, food, or pharmaceutical use.

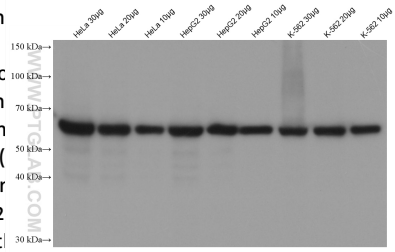
FAQ

1. Do I need to use this product for the entire duration of the blotting protocol?
A: No, it is not necessary. Just follow the recommended conditions for the steps using this product, and the steps not using this product should be done in the normal way.

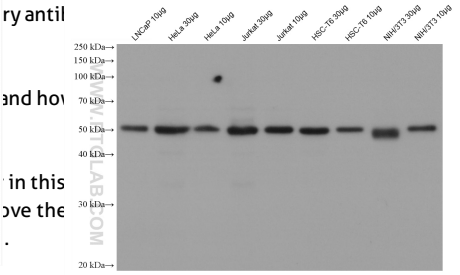
Validation Data



Various samples were loaded at 30 ug per well and subjected to Western blot, unblocked. Primary antibody 66444-1-Ig (Phospho-AKT (Ser473) Mouse Monoclonal Antibody) was diluted with PR20039 at 1:10,000 and incubated at room temperature for 15 minutes. The secondary antibody RGAM001 was diluted with PR20039 at 1:20,000 and incubated at room temperature for 10 minutes.



Various samples were loaded at different volumes per well and subjected to Western blot, unblocked. Primary antibody 15282-1-AP (HSP81 Rabbit Polyclonal Antibody) was diluted with PR20039 at 1:50,000 and incubated at room temperature for 18 minutes. The secondary antibody RGAR001 was diluted with PR20039 at 1:20,000 and incubated at room temperature for 8 minutes.



Various samples were loaded at different volumes per well and subjected to Western blot, unblocked. Primary antibody 66031-1-Ig (Alpha Tubulin Mouse Monoclonal Antibody) was diluted with PR20039 at 1:20,000 and incubated at room temperature for 3 minutes. The secondary antibody RGAM001 was diluted with PR20039 at 1:20,000 and incubated at room temperature for 3 minutes.

2. After the primary antibody reaction, directly soak the membrane in PBST/TBST washing solution and let it stand at room temperature as a stopping point (30 minutes to 4 hours is appropriate). When restarting the experiment, the membrane should be washed 3 times with washing solution for 1 minute each time.
Secondary antibody can be added after washing.
(2) After the secondary antibody reaction, soak the membrane directly in PBST/TBST wash solution and let it stand at room temperature as a stopping point (30 minutes to 4 hours is preferred). When restarting the

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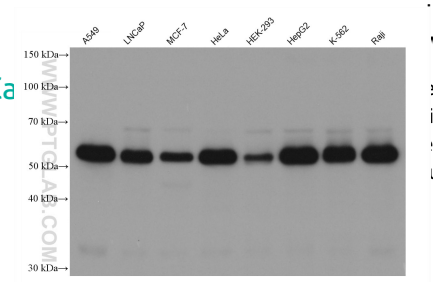
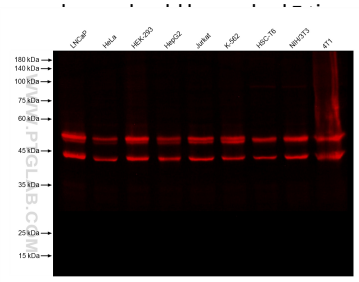
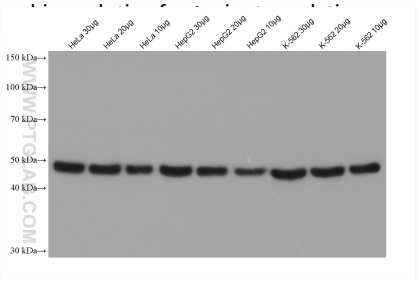


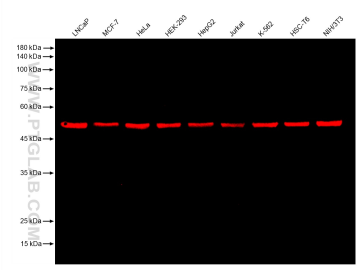
Various samples were loaded at 30 ug per well and subjected to Western blot, unblocked. Primary antibody 66184-1-Ig (p62 Mouse Monoclonal Antibody) was diluted with PR20039 at 1:20,000 and incubated at room temperature for 15 minutes. The secondary antibody RGAM001 was diluted with PR20039 at 1:20,000 and incubated at room... temperature for 10 minutes.



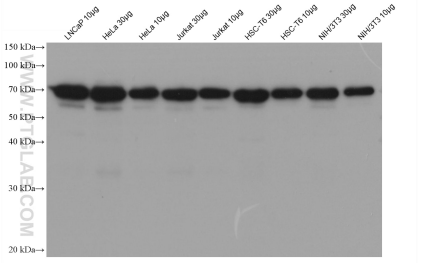
Various samples were loaded at 30 ug per well and subjected to Western blot, unblocked. Primary antibody 66210-1-Ig (JNK Mouse Monoclonal Antibody) was diluted with PR20039 at 1:10,000 and incubated at room temperature for 15 minutes. The secondary antibody RGAM006 was diluted with PR20039 at 1:10,000 and incubated at room... temperature for 10 minutes.



Various samples were loaded at different volumes per well and subjected to Western blot, unblocked. Primary antibody 80713-1-RR (Beta Tubulin Rabbit Recombinant Antibody) was diluted with PR20039 at 1:10,000 and incubated at room temperature for 18 minutes. The secondary antibody RGAR001 was diluted with PR20039 at 1:20,000 and incubated at... room temperature for 8 minutes.



Various samples were loaded at 30 ug per well and subjected to Western blot, unblocked. Primary antibody 80713-1-RR (Beta Tubulin Rabbit Recombinant Antibody) was diluted with PR20039 at 1:20,000 and incubated at room temperature for 15 minutes. The secondary antibody RGAR006 was diluted with PR20039 at 1:10,000 and incubated at... room temperature for 10 minutes.



Various samples were loaded at different volumes per well and subjected to Western blot, unblocked. Primary antibody HRP-66095 (HRP-conjugated Lamin B1 Mouse Monoclonal antibody) was diluted with PR20039 at 1:5,000 and incubated at room temperature for 3 minutes without a secondary antibody involved.

For technical support and original validation data for this product please contact

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