WB Rapid Antibody Diluent (Blocking-free)

Cat. No.: W1523001 | Pack size: 100ml | Storage: 2-8℃

Product Information

Item	Details
Available Pack Size(s)	100ml
Specifications & Purity	BioReagent, sterile, for western blot
Stability and Storage	Store at 2-8℃ long term (12 months).
Storage Conditions	Store at 2-8℃
Shipped In	Wet ice. This product requires cold chain shipping. Ground and other economy services are not available.

Product Description

This product is an innovative multifunctional antibody incubation working solution specially designed for Western Blot experiments. It integrates the functions of rapid reaction enhancement, background suppression and protein stabilization, and can be directly used for the dilution and incubation of primary and secondary antibodies, completely eliminating the independent blocking step. Its core feature allows users to complete incubation in only 1 hour at room temperature, obtaining detection signals and signal-to-noise ratio equivalent to or even better than those achieved with traditional diluents incubated overnight at 4℃. Meanwhile, it is fully compatible with the traditional overnight incubation method, greatly improving experimental flexibility and efficiency for researchers.

Product Principle

This incubation solution is based on an optimized physiologically compatible buffer system, supplemented with the following key components:

Reaction Enhancer: Promotes the specific binding of antibodies to antigens and significantly shortens the time required for binding equilibrium.

Multifunctional Background Inhibitor: Occupies non-specific sites on the membrane synchronously and dynamically during antibody incubation, achieving the effect of blocking during incubation and effectively reducing background noise.

Stabilizing Protective Agent: Maintains the activity of antibodies and the stability of antigen epitopes, ensuring reaction efficiency under rapid incubation conditions.

Core Advantages

1. Ultra-fast & High-efficiency: Complete primary antibody incubation in 1 hour at room temperature, saving over 90% of the time compared with the conventional overnight incubation (12-16 hours).
2. Superior Performance: The proprietary formula ensures rapid specific binding and low background. Experimental results meet or exceed the level of conventional overnight incubation in terms of signal intensity, specificity and band definition.
3. One-step Simplification: Eliminates the separate blocking step. It simplifies the three procedures of blocking, primary antibody incubation and secondary antibody incubation into two steps, shortening the overall experimental process by 2-3 hours and reducing operational errors.
4. Flexible & Compatible: Fully compatible with traditional experimental routines. Users can freely choose room-temperature rapid incubation (1 hour) or 4℃ overnight incubation according to sample requirements.
5. Cost-effective & Versatile: Suitable for all membrane-based Western Blot experiments (PVDF membrane, NC membrane). Compatible with primary and secondary antibodies derived from mouse, rabbit, goat and other species.

Experimental Materials Required

- Complete set of gel casting and electrophoresis supplies
- PVDF membrane or NC membrane with transferred protein
- Primary antibody to be tested
- Corresponding HRP-labeled (or other fluorescence-labeled) secondary antibody
- TBST or PBST washing buffer
- Orbital shaker (for room temperature and 4℃ use)
- Chemiluminescence imaging / developing system

Experimental Procedures

Note: The following procedures take PVDF membrane as an example. If using an NC membrane, the methanol activation step can be omitted.

Step 1: Membrane Transfer

Activate PVDF membrane with methanol. Equilibrate filter papers, sponges and gel in transfer buffer. Arrange components in the order from cathode to anode: sponge → filter paper → gel → membrane → filter paper → sponge. Remove air bubbles completely between layers, then perform membrane transfer as per laboratory conditions. Assess transfer efficiency via Ponceau S staining, and rinse with TBST for standby use.

Step 2: Primary Antibody Incubation (No Blocking Required)

Select either of the following protocols according to experimental requirements.

Protocol A: Rapid Incubation Method (Recommended for most proteins): 1. Fully immerse the membrane in an adequate volume of primary antibody working solution to ensure complete coverage. 2. Place on a shaker at room temperature and incubate with moderate shaking for 60 minutes.

Protocol B: Conventional Overnight Incubation Method (Recommended for very low-abundance proteins): 1. Prepare the primary antibody working solution as described above. 2. Immerse the membrane in the primary antibody working solution. 3. Place in a 4℃ cold room or refrigerator, and incubate overnight (12-16 hours) with low-speed shaking on a shaker.

Tip: The used primary antibody working solution can be recovered, stored at 4℃, and reused 2-3 times (with slightly reduced performance).

Step 3: Washing

Take the membrane out of the primary antibody working solution and place it in sufficient TBST. Wash rapidly on a shaker 3 times, 5 minutes each time.

Step 4: Secondary Antibody Incubation (No Blocking Required)

1. Prepare the secondary antibody working solution by diluting the HRP-conjugated secondary antibody with this incubation buffer at the dilution ratio recommended in the secondary antibody instructions (usually 1:5000 to 1:20000).
2. Immerse the washed membrane in the secondary antibody working solution.
3. Incubate on a shaker at room temperature with moderate shaking for 30 minutes.

Step 5: Final Washing and Detection

1. Take the membrane out of the secondary antibody working solution and place it in sufficient TBST. Wash on a shaker 3 times, 5 minutes each time.
2. Perform subsequent development and imaging operations according to the instructions of the selected detection substrate (such as ECL chemiluminescence solution).

Recommendations and Precautions

1. Antibody dilution: For first-time use, it is recommended to start with the standard dilution ratio stated in the antibody instructions. The dilution ratio can be adjusted later according to signal intensity.
2. Condition optimization: For the rapid incubation protocol, a slightly lower dilution ratio (e.g., 1.5-2 times lower dilution) can sometimes be adopted to enhance signal strength.
3. Low-abundance proteins: For target proteins with extremely low expression levels, if the rapid incubation method yields weak signals, prefer switching to 4℃ overnight incubation; then consider increasing the primary antibody concentration.
4. Membrane selection: This product is compatible with both PVDF and NC membranes. PVDF membrane features higher protein binding capacity and mechanical strength, and is the generally recommended choice.

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