One-step rapid WB (HRP) kit (mouse)

Project number: 0665505

Storage conditions: 2-8° C.

Products content

Component	066550550 preps
BlockingBuffer	500ml
AntibodyPretreatSolution (HRP/Mouse)	5 x 1ml
DilutionBuffer	500ml
WashBuffer (10×)	500ml

Product Introduction

One Step Rapid WB Kit (Mouse) is the newest WesternBlot kit developed by CombiVision, which can obtain high quality WesternBlot results in about 1 hour, with easy operation, high sensitivity, low background, no need to add secondary antibody, and high system stability. The conventional WesternBlot indirect assay process (blocking, primary antibody binding and secondary antibody binding) takes a long time, and the experimental process is complicated and requires multi-step condition optimization. After the protein on the gel is transferred to the carrier membrane, incubate the membrane with the blocking solution in the kit for 5 minutes, then incubate the carrier membrane with the primary antibody treated with the antibody reaction solution, and then wash the membrane three times (each time for 5 minutes), then luminescence or color development detection can be carried out. **This kit is intended for use in experimental systems where the primary antibody to the target protein is of murine origin.**

Matters needing attention

1. The client prepares his own primary antibody of murine origin.
2. Mix well before using BlockingBuffer Closure Solution, AntibodyPretreatSolution (HRP/Mouse) Antibody Reaction Solution (Mouse), and WashBuffer (10×) Rinse Solution.
3. If the rinsing solution is stored at 2-8℃ and precipitation occurs, please return to room temperature, dissolve the precipitation and use it normally, 1× rinsing solution can be stored at room temperature for one month.

4. It is recommended that the membrane be stained with a reagent such as Lichun red after the membrane transfer is completed and that the excess portion of the membrane be cut off to increase the efficiency of the reagents. 5. The primary antibody and the antibody reaction solution, HRP (Mouse), need to be pre-tested to determine the optimal amount of dilution.

6. The amount of antibody reaction solution HRP (mouse), antibody dilution solution and antibody can be scaled up or down proportionally to the size of the membrane.

7. Antibody dilutions with primary antibodies can be recovered and reused once. Antibodies with poor specificity and affinity are not recommended for reuse. If the recovered antibody is used within 1-2 days, please place it at 2-8℃, and for long-term storage, please freeze it at -20℃ to avoid repeated freezing and thawing.

8. If there is a high background, please adjust the amount of antibody and increase the number of membrane washes. 9. All reagents in the kit should be stored at 2-8℃, avoid freezing and thawing. 10.

Operation steps

This product is suitable for the sealing and antibody incubation steps after the completion of membrane transfer, taking 5cm×8cm membrane as an example:

1. Preparation of rinsing solution: Take 10 ml of WashBuffer (10×) and dilute it to 100 ml with distilled water, i.e. 1×WashBuffer, to be used. Use 8-10ml for each membrane wash.

2. Closure: After completion of membrane transfer, submerge the membrane into 10 ml BlockingBuffer and close it for 5 minutes at room temperature.

3. Rinsing: pour off the sealing solution, add 8-10 ml of 1×WashBuffer, and rinse on a shaker at a high speed for 1 minute. 4. Antibody incubation solution can be prepared at the same time as washing the membrane: Take 100 μl of AntibodyPretreatSolution (HRP/Mouse).

To the centrifuge tube, add 3-10 μg of primary antibody of murine origin, pipet until well mixed, and incubate for 5 minutes at room temperature. Add to 10 ml DilutionBuffer and mix well.

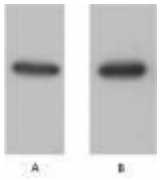
Note: 1) The dosage of primary antibody can also be adjusted according to the dilution of antibody. Take the final dilution of antibody 1:1000 as an example, take 100μl of antibody reaction solution HRP (mouse) into EP tube, add 10μl of primary antibody, add to 10ml of antibody dilution solution, mix well and incubate at room temperature for 5 minutes.

2) If the membrane area is small, the amount of antibody, reaction solution and diluent can be proportionally reduced.

5. After completing step 3, pour off the rinse solution and add the antibody incubation solution consisting of primary antibody, AntibodyPretreatSolution (HRP/Mouse) and DilutionBuffer to the membrane (make sure that the incubation solution completely submerges the surface of the membrane), and then incubate for 40 minutes on a shaker at room temperature at a speed of about 60 rpm.

6. Discard (recover) the antibody incubation solution and rinse 3-5 times with prepared 1×WashBuffer for 3 minutes each time. 7. Perform subsequent detection. ECL or DAB method is recommended for detection.

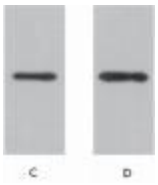
Examples of applications



Example 1 antigen is 293T cell whole lysate

A: Normal WB control: beta-actin murine monoclonal antibody 5μg incubated at room temperature for 40min, membrane washed secondary anti-sheep anti-mouse-HRP (CW0102) 1:10,000 dilution, room temperature for 40min, ECL exposure.

B:One-step WB: beta-actin murine monoclonal antibody 5μg incubated at room temperature for 40min, ECL exposure.



Example 2 antigen is E.coli multilabeled protein lysate

C: Normal WB control: GST mouse monoclonal antibody 2.5μg incubated at room temperature for 40 min, membrane washed and secondary anti-sheep anti-mouse-HRP 1:10,000 dilution, room temperature for 40 min, ECL exposure.

D: One-step WB: GST mouse monoclonal antibody 2.5ug incubated at room temperature for 40min, ECL exposure.

schedules

See Problems and Solutions

concern	Possible causes	prescription
Signal too weak or no band visible	Protein sample size too small	Increase the sample volume when performing SDS-PAGE electrophoresis.
	Protein transmembrane efficiency is too low	Optimize the transfer time or current to ensure that there are no air bubbles between the film and the adhesive when transferring the film.
	Lower affinity for primary antibodies	Increasing the incubation time of the membrane in solution or increasing the antibody concentration can increase the signal

	Lower affinity for primary antibodies	For low affinity antibodies, decreasing the time spent washing the membrane can increase the signal. From 10 minutes per wash, decreasing to 5 minutes per wash can increase signal.
High background	Primary antibody overdose	Reduce the amount of primary antibody used.
	Primary antibody binds non-specifically or cross-reacts with blocking reagents	Use serum consistent with the source of the secondary antibody or IgG-free BSA.
	Film washing time is too short	Adding a washing step can further reduce the background.
	Excessive exposure and development time	Reduce the exposure time. If both the signal and the background are high, wait a while for the background signal to diminish before exposing.
	Contaminated containers or reagents	Use a clean container for each wash. Wear gloves and use clean tweezers to handle the membrane.