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

Project number: 0665490

Storage conditions: 2-8℃.

Products content

Component	0665490 10preps	0665490 50preps
BlockingBuffer	100ml	500ml
AntibodyPretreatSolution (HRP/Rabbit)	1ml	5 x 1ml
DilutionBuffer	100ml	500ml
WashBuffer (10×)	100ml	500ml

Product introduction

One Step Rapid WB Kit (Rabbit) is the newest WesternBlot kit developed by KangWeiChi, which is able to obtain high-quality WesternBlot results in about 1 hour, with easy operation, high detection 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 min, then incubate the carrier membrane with primary antibody treated with antibody reaction solution, and then wash it three times (each time for 5 min), then it can be detected by luminescence or color development. **This kit is intended for use in experimental systems where the primary antibody of the target protein is of rabbit origin.**

Matters needing attention

1. Customers are required to prepare their own primary antibody of rabbit origin.
2. Please mix well before using BlockingBuffer Closure Solution, AntibodyPretreatSolution (HRP/Rabbit) Antibody Reaction Solution (Rabbit), 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 then 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 transfer is completed, and the excess part of the membrane be cut off to increase the efficiency of the reagent.
5. Primary antibody and antibody reaction solution HRP (rabbit) need to be pre-tested to determine the optimal amount of dilution.
6. Antibody reaction solution HRP (rabbit), antibody dilution solution and antibody dosage 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 low specificity and affinity are not recommended for reuse. If the recovered antibody is used within 1-2 days, please place it at 2-8℃, for long-term storage, please freeze it at -20℃, avoid repeated freezing and thawing.

8. If there is a higher 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.

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.

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/Rabbit) into a centrifuge tube, add 3-10 μg of rabbit primary antibody, and aspirate the antibody with a lance tip until it is well-mixed. mix well and incubate for 5 minutes at room temperature. Add to 10 ml DilutionBuffer and mix well.

Attention:

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 100 μl of antibody reaction solution HRP (rabbit) into EP tube, add 10 μl of primary antibody, add it to 10 ml of antibody dilution solution, mix well and incubate at room temperature for 5 minutes.

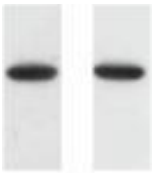
2) If the membrane area is small, the amount of antibody, reaction solution and diluent can be proportionally reduced.

5. After completing step 3, pour off the rinse solution and add the antibody incubation solution consisting of primary antibody, AntibodyPretreatSolution (HRP/Rabbit) 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 for 3 minutes each time with prepared 1 x WashBuffer.

7. Follow-up testing. It is recommended that the ECL or DAB method be used for testing.

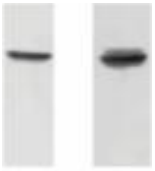
Application Example



Example 1 antigen is 293T cell whole lysate

A: Normal WB control: beta-actin rabbit polyantibody 3.3ug incubated at room temperature

for 40min, membrane washed secondary anti-goat anti-rabbit-HRP 1:10,000 dilution, room temperature for 40min, ECL exposure



Example 2 antigen is 293T cell whole lysate
C: Normal WB control: PAK1, Epitomics rabbit monoclonal antibody 1:1000 incubated at room temperature for 40 min, membrane washed secondary anti-goat anti-rabbit-HRP 1:10,000 dilution, room temperature for 40 min, ECL exposure
D: One-step WB: Epitomics rabbit monoclonal antibody 1:1000 incubated at room temperature for 40 min with ECL exposure.

Schedules

Frequently Asked Questions 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 10min per wash, reducing it to 5min per wash can increase the signal.
High background	Primary antibody overdose	Reduce the amount of primary antibody used.
	Primary antibody binds non-specifically	Use of serum from the same source as the secondary antibody or IgG-free BSA

	or cross-reacts with blocking reagents	
	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.