

## Rabbit Specific DAB Chromogenic Western Blot Kit – Blue – KOA0140

Rabbit Specific DAB Chromogenic Western Blot Kit is specific for detection of membrane-immobilized proteins on western blots probed with rabbit primary antibodies using HRP-conjugated anti-rabbit secondary antibodies and DAB chromogenic substrate reagents. The reaction product is a brown precipitate insoluble in water, dimethylbenzene or alcohol, which makes DAB suitable for color development reaction in western blotting. Rabbit DAB Chromogenic Reagent kit is extremely sensitive and has a high signal-to-noise ratio.

### I. KIT COMPONENTS

Rabbit Specific DAB Chromogenic Western Blot Kit - Blue			
Component	Catalog Number	Description	Size
Blocking Reagent	KOB0104	Blotto Blocking Buffer	2x10 g
Peroxidase Conjugated Secondary Antibody	KON0303	Anti-Rabbit IgG (Goat) Polymeric Peroxidase Conjugated Antibody	0.2 mL
Chromogenic Reagent	KOH0101	Three Component DAB Chromogenic Reagent (20X)	3x3 mL
Concentrated antibody diluent solution	KOI0101	Concentrated antibody diluent solution	20 mL

### II. STORAGE

Store at 4°C for one year. Avoid freezing.

### III. REQUIRED EQUIPMENT AND REAGENTS

- Nitrocellulose or PVDF membrane
- Diluent Buffer (pH 7.2~7.6)
  - Diluent Buffer (for preparation of blocking buffer and antibody solution): Add 2.42 g Tris, 9 g NaCl, 850-900 µl pure acetic acid into 1000 ml distilled water, adjust pH to 7.2-7.6.
- Wash Buffer
  - Add 0.5 ml of TWEEN 20 into 1000 ml of diluent buffer

### IV. PROTOCOL

Note: Rabbit IgG refers to the animal origin of the primary antibody, not the origin of the specimen. This Kit must be used on primary antibodies from rabbit.

1. Run protein sample and molecular weight standard through polyacrylamide gel electrophoresis (PAGE).
2. Transfer the protein sample to a nitrocellulose membrane or PVDF membrane.
3. Block membrane: Immerse the membrane in blocking buffer and incubate at room temperature for 1.5-2 hours or at 4°C overnight with agitation.
4. Wash membrane once for 10 minutes in Wash Buffer.

5. Incubate membrane with primary antibody: Dilute primary antibody in Diluent Buffer. Incubate membrane with primary antibody solution at room temperature for 2 hours 4°C overnight with agitation. Follow the antibody manufacturer's recommendations for optimized concentration.
6. Wash membrane in Wash Buffer with gentle agitation, 3 times for 10 minutes each.
7. Incubate the membrane with diluted secondary antibody at room temperature for 90 minutes or at 4°C overnight. Secondary antibody dilutions typically range from 1:2000-1:10000. Optimal secondary antibody dilutions must be determined empirically.
8. Wash membrane in Wash Buffer with gentle agitation, 4 times for 5 minutes each
9. Chemiluminescent Detection: Add 50µl chromogenic reagent A, reagent B, reagent C into 2 ml of distilled water and mix well. Add the working solution onto the membrane and incubate at room temperature until bands appear (usually 1-5 minutes). Wash the membrane with distilled water to stop the reaction.
10. Observe the bands and take pictures.

## V. TROUBLESHOOTING

### Weak or No Signal

Possible Cause	Solution
<b>Improper protein transfer to membrane</b>	Stain gel after transfer is complete to determine transfer is efficient Use Ponceau S to stain membrane to determine transfer is efficient Ensure sufficient contact between gel and membrane during transfer Make sure transfer sandwich is assembled correctly Wet membrane according the instruction Avoid overheating during electro-transfer Use positive control or molecular weight markers Optimize transfer time and current Avoid sample (antigenic determinant) destroy when handling
<b>Insufficient protein and membrane binding</b>	Adding 20% methanol to transfer buffer Use small-bore membrane
<b>Insufficient antibody</b>	Increase antibody concentration
<b>Insufficient antigen</b>	Load more protein
<b>Antigen masking by blocking buffer</b>	Compare different blocking buffers Optimize protein concentration of blocking agent Reduce blocking time
<b>Presence of sodium azide in buffers</b>	Eliminate sodium azide from buffers
<b>Too short exposure time</b>	Lengthen film exposure time
<b>Too short substrate incubation time</b>	Lengthen substrate incubation time to five minutes
<b>Digestion of protein on membrane</b>	Optimize amount of blocking agent
<b>Degradation of protein during storage</b>	Re-prepare protein sample
<b>Incompatible primary and secondary antibodies</b>	Make sure primary antibody, secondary antibody, substrate, enzyme system and samples are compatible Use loading control to test effectiveness of second detecting system
<b>Low concentration of primary antibody and/or secondary antibody</b>	Increase antibody concentration Increase incubation time
<b>Cross-reactivity between blocking agent and antibodies (primary or secondary)</b>	Use mild detergent such as Tween20 Change blocking agent (commonly used are milk, BSA, serum or gelatin)

<b>Inability of primary antibody to recognize the protein in tested sample</b>	Check instruction Use positive control
<b>Low or none content of target protein (ineffective antigen)</b>	Use positive control Increase loading amount to 20-30 µg protein per well Use protease inhibitor or fractional extract target protein
<b>Insufficient transfer and excessive wash</b>	Check the transfer with Ponceau S Soak PVDF-membrane in methanol Avoid excessive wash
<b>Over-blocking</b>	Use 0.05% skim milk or no milk diluents buffer Change blocking agent Reduce blocking time
<b>Loss of primary antibody effectiveness</b>	Prepare fresh antibody and store properly when not in use Avoid repeated freezing and thawing
<b>Inhibition of secondary antibody by sodium azide</b>	Avoid using sodium azide together with HRP- conjugated antibodies
<b>Loss of effectiveness in enzyme conjugate and substrate</b>	Mix enzyme conjugate and substrate (no color development when enzyme is inactive) Use activated enzyme conjugate and fresh substrate
<b>Improper wet transfer for membrane</b>	Soak PVDF membrane in 100% methanol
<b>Insufficient molecular weight of target protein (&lt; 10 kDa)</b>	Use small-bore membrane Reduce transfer time
<b>Equality or nearness in values between target protein's isoelectric point and transfer buffer's pH value</b>	Try other buffers such as CAPS buffer (pH 10.5) Try low pH value buffers such as acetic acid buffer
<b>Too high methanol concentration</b>	Decrease methanol concentration or use isopropyl alcohol

## High Background

Possible Cause	Solution
<b>Too high antibody concentration</b>	Optimize and decrease antibody concentration
<b>Aggregate secondary antibody formation</b>	Filter the secondary antibody through 0.2µm filter Use a new secondary antibody
<b>Too high antibody incubation temperature</b>	Incubate the antibody at 4°C
<b>Non-specific secondary antibody binding or cross-reactivity with blocking agent</b>	Run secondary antibody control (without the primary) Decrease secondary antibody concentration
<b>Cross-reactivity of primary or secondary antibody with blocking agent</b>	Add Tween-20 to the incubation and washing buffer
<b>Incompatible blocking agent</b>	Compare different blocking buffers
<b>Incomplete blocking</b>	Optimize choice of blocking buffer Increase protein concentration in blocking agent Optimize blocking time and/or temperature; Block for 2 hours at normal temperature or overnight at 4°C Add 0.05% Tween 20 detergent into blocking agent Add 0.05% Tween 20 detergent into antibody diluents solution
<b>Insufficient blocking</b>	Extend blocking time or use a compatible blocking agent (e.g. skim milk, BSA, serum, etc.)
<b>Cross-reactivity of antibody with other proteins</b>	Use different blocking agent (Do not use skim milk with biotin system) Reduce secondary antibody concentration Test cross-reactivity between secondary antibody and membrane

<b>Insufficient washing</b>	Increase number of washes and buffer volume Add 0.05% Tween 20 detergent into washing buffer
<b>Too long exposure time</b>	Reduce exposure time
<b>Membrane problem</b>	Use clean tweezers; Operate with gloves Use new membranes Ensure the liquid is enough to keep the membrane moist Use decolorization table in incubation Avoid membranes overlapping Handle carefully and avoid damaging membrane
<b>Insufficient membrane wash</b>	Increase the number of wash
<b>Incompatible membrane</b>	Nitrocellulose membrane's background is lower than that of PVDF membrane
<b>Dry membrane</b>	Make sure membrane is covered with enough liquid and prevent it from drying
<b>Contaminated buffer</b>	Use new buffer or filter buffer before use
<b>Contaminated equipment</b>	Ensure all equipment and tools are clean and no gel is left on membrane

## VI. RELATED PRODUCTS

Component	Catalog #	Size
10x TBS pH 7.5	<a href="#">MB-012</a>	1000 ml
10x TTBS pH 7.5	<a href="#">MB-013</a>	1000 ml
10X PBS pH 7.2	<a href="#">MB-008</a>	1000 ml
10x PBST pH 7.2	<a href="#">MB-075-1000</a>	1000 ml