Rat Specific DAB Chromogenic Western Blot Kit - Yellow

Rat Specific DAB Chromogenic Western Blot Kit is specific for detection of membrane-immobilized proteins on western blots probed with rat primary antibodies using HRP-conjugated anti-rat 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. Rat DAB Chromogenic Reagent kit is extremely sensitive and has a high signal-to-noise ratio.

I. KIT COMPONENTS

Rat Specific DAB Chromogenic Western Blot Kit - Yellow			
Component	Catalog Number	Description	Size
Blocking Reagent	KOB0104	Blotto Blocking Buffer	2x10 g
Peroxidase Conjugated Secondary Antibody	KON0304	Anti-Rat IgG (Rabbit) Polymeric Peroxidase Conjugated Antibody	0.2 mL
Chromogenic Reagent	KOH0101	Three Component DAB Chromogenic Reagent (20X)	3x3 mL
Antibody Diluent Solution	KOI0101	Antibody Diluent (10X)	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: Rat 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 rat.

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, and 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 30 seconds-5 minutes). Note: It is better to prepare the working solution just before use. 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
Improper protein transfer to membrane	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
Insufficient protein and membrane binding	Adding 20% methanol to transfer buffer Use small-bore membrane
Insufficient antibody	Increase antibody concentration
Insufficient antigen	Load more protein
Antigen masking by blocking buffer	Compare different blocking buffers Optimize protein concentration of blocking agent Reduce blocking time
Presence of sodium azide in buffers	Eliminate sodium azide from buffers
Too short exposure time	Lengthen film exposure time
Too short substrate incubation time	Lengthen substrate incubation time to five minutes
Digestion of protein on membrane	Optimize amount of blocking agent
Degradation of protein during storage	Re-prepare protein sample
Incompatible primary and secondary antibodies	Make sure primary antibody, secondary antibody, substrate, enzyme system and samples are compatible Use loading control to test effectiveness of second detecting system

Low concentration of primary antibody and/or secondary antibody	Increase antibody concentration Increase incubation time
Cross-reactivity between blocking agent and antibodies (primary or secondary)	Use mild detergent such as Tween20 Change blocking agent (commonly used are milk, BSA, serum or gelatin)
Inability of primary antibody to recognize the protein in tested sample	Check instruction Use positive control
Low or none content of target protein (ineffective antigen)	Use positive control Increase loading amount to 20-30 µg protein per well Use protease inhibitor or fractional extract target protein
Insufficient transfer and excessive wash	Check the transfer with Ponceau S Soak PVDF-membrane in methanol Avoid excessive wash
Over-blocking	Use 0.05% skim milk or no milk diluents buffer Change blocking agent Reduce blocking time
Loss of primary antibody effectiveness	Prepare fresh antibody and store properly when not in use Avoid repeated freezing and thawing
Inhibition of secondary antibody by sodium azide	Avoid using sodium azide together with HRP-conjugated antibodies
Loss of effectiveness in enzyme conjugate and substrate	Mix enzyme conjugate and substrate (no color development when enzyme is inactive) Use activated enzyme conjugate and fresh substrate
Improper wet transfer for membrane	Soak PVDF membrane in 100% methanol
Insufficient molecular weight of target protein (< 10 kDa)	Use small-bore membrane Reduce transfer time
Equality or nearness in values between target protein's isoelectric point and transfer buffer's pH value	Try other buffers such as CAPS buffer (pH 10.5) Try low pH value buffers such as acetic acid buffer
Too high methanol concentration	Decrease methanol concentration or use isopropyl alcohol

High Background

Possible Cause	Solution
Too high antibody concentration	Optimize and decrease antibody concentration
Aggregate secondary antibody formation	Filter the secondary antibody through 0.2µm filter Use a new secondary antibody
Too high antibody incubation temperature	Incubate the antibody at 4°C
Non-specific secondary antibody binding or cross-reactivity with blocking agent	Run secondary antibody control (without the primary) Decrease secondary antibody concentration
Cross-reactivity of primary or secondary antibody with blocking agent	Add Tween-20 to the incubation and washing buffer
Incompatible blocking agent	Compare different blocking buffers

Incomplete blocking	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
Insufficient blocking	Extend blocking time or use a compatible blocking agent (e.g. skim milk, BSA, serum, etc.)
Cross-reactivity of antibody with other proteins	Use different blocking agent (Do not use skim milk with biotin system Reduce secondary antibody concentration Test cross-reactivity between secondary antibody and membrane
Insufficient washing	Increase number of washes and buffer volume Add 0.05% Tween 20 detergent into washing buffer
Too long exposure time	Reduce exposure time
Membrane problem	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
Insufficient membrane wash	Increase the number of wash
Incompatible membrane	Nitrocellulose membrane's background is lower than that of PVDF membrane
Dry membrane	Make sure membrane is covered with enough liquid and prevent it from drying
Contaminated buffer	Use new buffer or filter buffer before use
Contaminated equipment	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	MB-012	1000 ml
10x TTBS pH 7.5	MB-013	1000 ml
10X PBS pH 7.2	MB-008	1000 ml
10x PBST pH 7.2	MB-075-1000	1000 ml