DAB Peroxidase Substrate Kit

BiOri BIOTECH

Product Description

DAB Peroxidase Substrate Kit is a colorimetric substrate kit developed for horseradish peroxidase (HRP) detection. The kit contains all reagents required for HRP detection and is suitable for staining PVDF and NC membranes used in Western blot assays. DAB (3,3'-diaminobenzidine tetrahydrochloride) is a commonly used substrate for HRP. In the presence of HRP, DAB is oxidized to form a brown precipitate that is insoluble in water and ethanol. HRP immobilized at the target protein site after immunoreaction catalyzes the conversion of DAB into a brown reaction product, allowing the location and expression level of the target protein to be assessed based on the color reaction.

Components

Components	BR4D601-01 60 mL
20×DAB-A	3 mL
1×DAB-B	60 mL

Storage

Store at 2-8°C protected from light.

Notes

1. For Research Use Only. Not for use in diagnostic procedures.
2. DAB may be carcinogenic. Handle with care and avoid direct contact.
3. Use a sufficient volume of working solution to ensure complete coverage of the blotting membrane.
4. If background staining is too strong after color development, consider extending the blocking time to reduce non-specific staining or shortening the DAB development time. If staining is weak or absent, consider increasing the concentration of the primary or secondary antibody or extending the development time appropriately.
5. For your safety and health, please wear a lab coat and disposable gloves when operating.

Protocol

1. Prepare the complete DAB working solution by adding 50 μL of 20×DAB-A to each 1 mL of 1×DAB-B and mix well. Protect the prepared working solution from light and use within 30 min.
2. Add the working solution evenly to the membrane and incubate at room temperature protected from light for 1-30 min. If no background appears, development may be continued. After development, wash the membrane with distilled water to stop the reaction.
3. Observe and photograph the membrane.

Troubleshooting and FAQs

1. Background staining is too strong

Insufficient blocking: Use of an unsuitable blocking reagent or insufficient blocking time can increase non-specific binding and deepen background staining. Use an appropriate blocking reagent or extend the blocking time.

Non-specific antibody adsorption: Inappropriate antibody selection can cause non-specific adsorption. Use high-quality primary and secondary antibodies to reduce non-specific binding.

Overdevelopment or excessive secondary antibody concentration: Excessive development time or overly high secondary antibody concentration can increase non-specific staining. Shorten the development time or reduce the secondary antibody concentration.

Insufficient washing: Incomplete washing can leave residual antibodies or impurities that react with DAB and deepen background staining. Use a wash buffer of appropriate strength or extend the washing time.

2. No staining or weak staining

Antibody concentration is too low: Low primary or secondary antibody concentration reduces antigen binding and weakens the signal. Increase the concentration appropriately and verify secondary antibody performance if necessary.

Detection system is insufficiently sensitive: The current detection system may not be sensitive enough to detect low-expression antigens. Consider using a more sensitive detection system or increasing the sample loading amount.

Development time is too short: Insufficient development time leads to incomplete reaction between DAB and HRP and therefore weak staining. Extend the development time appropriately.

3. Staining is uneven

Uneven reagent distribution: If the reagent does not completely cover the membrane, the reagent at the edges may dry first and become more concentrated than at the center, causing darker staining. Bubbles can also cause uneven staining. After adding the reagent, verify that it is evenly distributed and that the entire membrane is fully covered.

DAB staining solution is not mixed thoroughly: Uneven DAB concentration on the membrane can cause uneven staining. Mix the staining solution thoroughly, and after adding it, gently rock the membrane back and forth so the substrate concentration remains uniform across the membrane.