

1. Safety warnings and precautions

Correct operations are necessary. Safety warnings and precautions of this product. The complete instructions should be read and fully understood before attempting to use the product.

The Procedure described in the instruction manual applies only to the use for the intended purpose. Using the product for any purpose other than the intended use or in any manner other than that described in the manual is forbidden.

User shall be liable for all safety measures needed for any use other than specified in the manual.

2. Introduction

EzWestBlue is a chromogenic substrate for detection of antibodies labeled with horseradish peroxidase (HRP) on western blotting. This product cannot be used as a chromogenic substrate in ELISA.

3. Package

EzWest Blue (200mL).....1 Bottle

* One bottle serves for 2000 cm² of blotting membrane (approximately 26 sheets of mini-gel-size membrane)

4. Components

EzWestBlue is ready to use. No addition of or mixing with other reagents is necessary.

- 3, 3', 5, 5'-tetramethylbenzidine
- Hydrogen peroxide

EzWestBlue is a clear, colorless or slightly blue solution.

5. Procedure

1. Sufficiently wash the blotting membrane reacted with HRP-conjugated antibodies with wash buffer. Incomplete wash may cause high background.

2. Warm EzWestBlue to room temperature, in advance. The required volume of the product is 100 μ L per 1 cm² of transfer membrane.

3. Place the washed transfer membrane in a clean tray and apply an appropriate amount of EzWest Blue to the membrane. Make sure that the whole membrane is covered with the solution.

* Shaking may disturb signals or reduce sensitivity. The tray must be placed stationarily.

4. Reaction time is dependent on sample. The bands generally become visualized in blue within 5 - 15 minutes. Long reaction time may cause high background.

5. To stop the reaction, wash the blotting membrane with a large amount of distilled water. Repeat the wash step several times. The use of acids for washing discolors bands yellow, which may become difficult to see on a white membrane. So, the termination of reaction must be achieved by using distilled water.

6. The blotting membrane after reaction should be stored avoiding light, in a tightly closed plastic bag. Even during appropriate storage, the color of signals is fading. Immediate scanning of the membrane with a scanner is recommended.

6. Storage

· EzWestBlue should be stored in a refrigerator, avoiding light. Unopened reagent is stable until mentioned expiration date.

· EzWestBlue turns brown through contact with metal ions. Prevent EzWestBlue from being contaminated by tap water, etc.



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