INSTRUCTIONS

# SuperSignal® Western Blot Enhancer

46640 46641 2265.1

| Number | Description |
|---|---|
| **46640** | **SuperSignal Western Blot Enhancer,** sufficient material for 25 mini blots or 2000cm$^2$ of membrane<br>**Kit Contents:**<br>**Antigen Pretreatment Solution,** 250mL<br>**Primary Antibody Diluent,** 250mL |
| **46641** | **SuperSignal Western Blot Enhancer,** sufficient material for two mini blots or 160cm$^2$ of membrane<br>**Kit Contents:**<br>**Antigen Pretreatment Solution,** 25mL<br>**Primary Antibody Diluent,** 25mL |
| | **Storage:** Upon receipt store at 4°C. Product is shipped at ambient temperature. |

## Introduction

The Thermo Scientific SuperSignal Western Blot Enhancer increases both signal intensity and sensitivity 3- to 10-fold compared to detection performed using conventional Western blotting. When a protein or antigen is difficult to detect because of low abundance or poor immunoreactivity, the SuperSignal Western Blot Enhancer can significantly reduce background and enhance detection of low abundant and weakly immunoreactive antigens. This versatile kit works with PVDF and nitrocellulose membranes and is compatible with fluorescence, chromogenic and chemiluminescent detection.

## Important Product Information

- Apply the Antigen Pretreatment Solution to the membrane after protein transfer and before applying blocking buffer.
- Use the Primary Antibody Diluent to dilute the primary antibody.
- Use any blocking solution to prepare appropriate dilution of secondary antibody conjugate.

## Additional Materials Required

- Ultrapure water
- Nitrocellulose or PVDF membrane with transferred proteins
- Appropriate primary antibody and secondary antibody conjugate
- Wash Buffer: phosphate- or Tris-buffered saline containing 0.05% Tween®-20 Detergent
- Blocking Buffer (e.g., Thermo Scientific SuperBlock Blocking Buffer, Product No. 37515 or 37535)
- Shaker for membrane incubations

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## Procedure for Western Blot Enhancer Treatment

**Important Note:** For all steps, use sufficient volumes to completely immerse the membrane. Enhancer treatment must be completed before performing membrane blocking.

1. Perform electrophoresis and transfer protein onto a nitrocellulose or PVDF membrane.
2. Wash the membrane with ultrapure water for two minutes with shaking.
3. Discard the water and rinse the membrane twice with ultrapure water.
4. Add sufficient volume of Antigen Pretreatment Solution to immerse the membrane. Incubate at room temperature for 10 minutes with shaking.
5. Discard the solution from the tray. Rinse the membrane five times with ultrapure water.
6. Incubate the membrane with Blocking Buffer.
7. Rinse the membrane with Wash Buffer three times. Wash the membrane with Wash Buffer for 5 minutes with shaking.
8. Dilute the primary antibody in Primary Antibody Diluent. Use an antibody concentration appropriate for the specific detection method. See Table 1 for concentration ranges for chemiluminescent substrates.
9. Incubate the membrane in the diluted primary antibody for 1 hour at room temperature with shaking.
10. Wash the membrane with Wash Buffer four times for 5 minutes each.
11. Dilute the secondary antibody conjugate in Blocking Buffer. Use an antibody concentration appropriate for the specific detection method. See Table 1 for concentration ranges for chemiluminescent substrates.
12. Incubate the membrane in the diluted secondary antibody conjugate for 30 minutes at room temperature with shaking.
13. Wash the membrane with Wash Buffer four times for 5 minutes each.
14. Detect protein with the appropriate detection system.

**Table 1. Primary and secondary antibody concentrations to use with Thermo Scientific Chemiluminescent Substrates.**

| Substrate | Pierce ECL | SuperSignal West Pico | SuperSignal West Dura | SuperSignal West Femto |
|---|---|---|---|---|
| **Primary Antibody Concentration (µg/mL)** | 0.2-10 | 0.2-1.0 | 0.02-1.0 | 0.01-0.2 |
| **Secondary Antibody Concentration (ng/mL)** | 67-1000 | 10-50 | 4-20 | 2-10 |

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Weak or no signal | Primary or secondary antibody concentration is too low | Use more primary or secondary antibody |
| | Too much enzyme in the system depleted the chemiluminescent substrate and caused the signal to fade quickly | Use less secondary antibody |
| | Inefficient protein transfer | Optimize transfer conditions |
| High background | Inadequate blocking and/or washing | Optimize blocking buffer and washing steps |
| | Secondary antibody conjugate concentration is too high | Use less secondary antibody conjugate |

## Additional Information

**Visit the website for additional information relating to this product including the following:**

- Request your free copy of the Western Blotting Handbook, which contains a large troubleshooting section, detailed Western blotting protocols and dozens of SuperSignal Substrate references
- Tech Tip # 24: Optimize antigen and antibody concentrations for Western blots
- Tech Tip # 67: Chemiluminescent Western blotting technical guide and protocols
- Tech Tip # 21: Convert to SuperSignal West Pico Substrate from ECL

## Related Thermo Scientific Products

| | |
|---|---|
| **26681** | **Pierce Blue Prestained Protein Molecular Weight Marker Mix,** 1 × 48 microtube plate |
| **34080** | **SuperSignal West Pico Chemiluminescent Substrate,** 500mL |
| **34076** | **SuperSignal West Dura Extended Duration Substrate,** 200mL |
| **34096** | **SuperSignal West Femto Maximum Sensitivity Substrate,** 200mL |
| **32106** | **Pierce ECL Western Blotting Substrate,** 500mL |
| **37515** | **SuperBlock Blocking Buffer in PBS,** 1L |
| **37535** | **SuperBlock Blocking Buffer in TBS,** 1L |
| **21059** | **Restore™ Western Blot Stripping Buffer,** 500mL |
| **21065** | **Pierce Background Eliminator Kit,** for eliminating background from X-ray film |
| **34090** | **CL-XPosure™ Film, 5" × 7" sheets,** 100 sheets/pkg |

### General Reference

Alegria-Schaffer, A., *et al*. (2009). Performing and optimizing Western blots with an emphasis on chemiluminescent detection. *Methods Enzymol* **463:**573-99.

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