

# Western Blot Signal Enhancer – Nanograde

Cat#: A1001 250 ml

Storage: Room Temp.

A1002 500 ml

## INTRODUCTION

**Western Blot Signal Enhancer – Nanograde** (abbrev. for WB Signal Enhancer) is a protein-free, chemical-defined, Nano grade, one-reagent system for enhancing desired antigen-antibody interactions while reducing nonspecific background signal. This reagent is designed for low-affinity antibodies, low-expression and phosphorylated protein detection. Without blocking step, simply dilute 1<sup>st</sup> and 2<sup>nd</sup> antibodies in WB Signal Enhancer, no requiring any additional steps, and therefore WB Signal Enhancer is an ideal reagent for enhancing signal and reducing background in Western blot experiment.

## CHARACTERISTICS

### 1. **NO need blocking step**

After transfer step, skip the blocking step and directly incubate membrane in 1<sup>st</sup> antibody which is diluted with WB Signal Enhancer.

### 2. **Enhance specific signal while reducing background**

WB Signal Enhancer strengthens the specific antigen-antibody reaction, while reducing background.

### 3. **Ready to use**

WB Signal Enhancer is formulated as ready-to-use, no diluting necessary. Simply replace your antibody dilution buffer with WB Signal Enhancer.

### 4. **For phosphorylated protein**

Without protein interference, WB Signal Enhancer is suitable for phosphorylated protein detection.

<b>Important:</b> 1. DO NOT need blocking step. 2. Please use WB Signal Enhancer in all dilution steps.
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## PROTOCOL

1. After transfer step, rinse the PVDF/NC membrane with the PBS for 1-3 mins.
2. Dilute the 1<sup>st</sup> antibody with **WB Signal Enhancer** and incubate the membrane at room temperature for 1-2 hours.

Note: (1) Please **DO NOT** incubate over 4 hours to overnight; this will cause the high background.

(2) The primary antibody can be reused for one or two times.

3. Wash the membrane with PBST/TBST for three times.
4. Dilute the 2<sup>nd</sup> antibody with WB Signal Enhancer and incubate the membrane at room temperature for 1 hour.
5. Wash the membrane with PBST/TBST for three times.
6. Perform the image development methods (ECL / NBT).

## **TROUBLESHOTTING**

Problem	Possible reason	Suggestion
High Background	The concentration of 2 <sup>nd</sup> antibody was too high	Recommended dilution fold between 1:10000-1:20000
	Insufficient Blocking or 2 <sup>nd</sup> antibody dilute in only PBST/TBST	Suggest dilute antibodies with WB Signal Enhancer or BSA in PBST/TBST
	Incubation time too long	Appropriate incubation time is 1-2 hrs and <b>DO NOT</b> exceed 4hrs.
Weak signal	1 <sup>st</sup> or 2 <sup>nd</sup> antibody concentrations too low	Optimize the dilution fold, followed by manufacture's instruction
Sticky or gel aggregation	Normal condition. Because nanoparticle would aggregate when encountering low temperature.	Return solution into Room temp.

## **PRODUCT USE LIMITATION**

These products are intended for research use only.