

Antibody Signal Enhancer

<u>Code</u>	<u>Description</u>	<u>Size</u>
M336-200ML	Antibody Signal Enhancer <i>Sufficient for 10 blots</i>	200 ml
M336-500ML	Antibody Signal Enhancer <i>Sufficient for 25 blots</i>	500 ml
M336-1L	Antibody Signal Enhancer <i>Sufficient for 50 blots</i>	1 L

General Information:

Antibody Signal Enhancer is a Western blotting antibody diluent that increases intensity of protein detection up to 5-fold compared to conventional blotting. The enhancer is a ready-to-use solution that replaces TBS-T with non-fat dry milk, or other diluent, for antibody incubation steps. It is formulated for use with HRP- and biotin-conjugated antibodies and works with both PVDF and nitrocellulose blots. Antibody Signal Enhancer is stable at room temperature and is a non-hazardous formulation.

- Improve detection of low abundance proteins
- Conserve valuable antibody reagents
- No extra incubation steps required

Storage/Stability:

Store at room temperature (18°C - 26°C).

Application Disclaimer

*For research use only.
Not for therapeutic or diagnostic use.*



Procedure

1. Separate proteins by electrophoresis and then transfer proteins onto PVDF or nitrocellulose.
2. Block the membrane with an appropriate blocking solution, such as TBS-T/5% non-fat dry milk or Rapid Block™ (M325).
3. Dilute the primary antibody using Antibody Signal Enhancer. Optimal antibody concentration and incubation time are to be determined empirically, but it is recommended to initially use the antibody supplier's recommendation.
4. Incubate the membrane in the antibody solution for 1 hour or more with shaking at room temperature, or overnight at 4°C.
5. Wash the membrane in TBS-T or PBS-T for 5 minutes with shaking. Repeat the washing step two additional times.
6. Dilute the secondary antibody using Antibody Signal Enhancer. Optimal antibody concentration and incubation time are to be determined empirically, but it is recommended to initially use the antibody supplier's recommendation.
7. Incubate the membrane in the antibody solution for 1 hour at room temperature with shaking.
8. Wash the membrane in TBS-T or PBS-T for 5 minutes with shaking. Repeat the washing step two additional times.
9. Detect protein with an appropriate detection system, such as VisiGlo Plus™ HRP Chemiluminescent Substrate Kit (N219) or VisiGlo Plus™ AP Chemiluminescent Substrate (N217).

Frequently Asked Questions

Questions	Answers
Which type of secondary antibody should be used?	A secondary antibody conjugated to HRP, biotin, or alkaline phosphatase.
Is Antibody Signal Enhancer compatible with fluorescent blotting?	This product has not been optimized for use with secondary antibodies conjugated to fluorophores.
Why don't I see a 5-fold increase in antibody signal?	Enhancement ranges typically from 1.5- up to 5-fold compared to standard blotting. The extent of the enhancement varies between different antibody-antigen pairs.
Why do I see extra bands?	1- Antibody may be too concentrated. Try serial dilutions of antibody to find optimal concentration. 2 - Excess protein on the blot. Serially dilute proteins to find optimal concentration. 3- Inadequate blocking. Test blotting with other blocking conditions. 4- Insufficient washing. Increase wash volume, frequency, and/or duration.
Why is the signal on my blot weak?	1- Antibody may be too dilute. Try serial dilutions of antibody to find optimal concentration. 2- Incomplete transfer. Optimize transfer time and electric current. 3. Insufficient protein on gel. Try loading serial dilutions of protein on gel.
Why do I see white bands on my blot?	Too much antibody and/or antigen, which depletes chemiluminescent substrate quickly, leaving areas of negative staining. Try reducing antibody and/or antigen.
Can antibody stored in Antibody Signal Enhancer be reused?	Store for reuse up to 1 week at 4°C, although freshly prepared antibody solutions yield best results.
Is Antibody Signal Enhancer compatible with blocking using RapidBlock™ Solution?	Yes. After blocking with RapidBlock™, proceed immediately to incubation with primary antibody diluted in Antibody Signal Enhancer. There is no need to wash between these steps.



Product Information

Related Products

Code	Product
M325-15ML	Rapid Block™ Solution, 10X
M235-5PK	TBS with Tween® 20
N218-KIT	VisiGlo™ HRP Chemiluminescent Substrate Kit
N219-KIT	VisiGlo Plus™ HRP Chemiluminescent Substrate Kit

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