

Clean-Blot™ IP Detection Reagent (HRP)

21230

2036.2

Number	Description
21230	Clean-Blot IP Detection Reagent (HRP), 2.5 ml (40 µg/ml)

Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Note: If using mouse IgG₁, perform a dot blot to determine compatibility. Clean-Blot IP Detection Reagent (HRP) might not detect mouse IgG₁.

Introduction

Clean-Blot IP Detection Reagent (HRP) enables Western blot detection of target proteins without interference from denatured IgG. Background signal that can mask the target protein band is often caused by heavy and light chains from IgGs (~50 and 25 kDa). Western blot interference from IgGs results from immunoprecipitation methods that release antibody with the antigen and samples types that contain IgGs, such as tissue extracts (e.g., liver, spleen). Secondary antibodies then detect the denatured and blotted IgGs. Clean-Blot IP Detection Reagent (HRP), however, detects only the native antibodies, providing accurate and specific detection of the target antigen.

Procedure for Western Blotting

A. Additional Materials Required

- Western blot on nitrocellulose or PVDF membrane
- Blocking buffer such as StartingBlock™ T20 Blocking Buffer (TBS, Product No. 37543 or PBS, Product No. 37539), SuperBlock® T20 Blocking Buffer (TBS, Product No. 37536 or PBS, Product No. 37516), or 1-5% dry milk
- Wash buffer such as Tris-buffered saline (TBS, Product No. 28376) or phosphate-buffered saline (PBS, Product No. 28372) containing 0.05% Tween®-20 (Product No. 28320)
- Primary antibody specific for antigen of interest
- Horseradish peroxidase (HRP) substrate
- Film or CCD camera if using a chemiluminescent substrate

B. Antibodies Compatible with Clean-Blot IP Detection Reagent

The Clean-Blot IP Detection Reagent detects the polyclonal and monoclonal antibodies listed in the table below. For best results, test each specific antibody.

<u>Species</u>	<u>Monoclonal Isotype(s)</u>
Bovine	IgG ₂
Goat	IgG ₂
Human	IgG ₁ , IgG ₂ , IgG ₄
Mouse	IgG _{2a} , IgG _{2b} , IgG ₃
Rat	IgG _{2c}
Rabbit	Total IgG
Sheep	IgG ₂

C. Concentration Guidelines for the Clean-Blot IP Detection Reagent

Note: These dilution ranges are guidelines. For best results, optimize the dilution for each specific experiment.

HRP Substrate	Clean-Blot IP Detection Reagent Dilution Range
SuperSignal [®] West Femto Chemiluminescent Substrate	1:200 to 1:4,000 (e.g., for 1:4,000 dilution, add 2.5 µl of detection reagent to 10 ml of blocking buffer)
SuperSignal West Dura Chemiluminescent Substrate	1:200 to 1:2,000 (e.g., for 1:2,000 dilution, add 5 µl of detection reagent to 10 ml of blocking buffer)
SuperSignal West Pico Chemiluminescent Substrate	1:40 to 1:1,000 (e.g., for 1:1,000 dilution, add 10 µl of detection reagent to 10 ml of blocking buffer)
Pierce [®] ECL Western Blotting Substrate	1:40 to 1:400 (e.g., for 1:400 dilution, add 25 µl of detection reagent to 10 ml of blocking buffer)

D. Western Blot Detection

1. Dilute primary antibody to appropriate concentration in blocking buffer.

Note: If using mouse IgG₁, perform a dot blot to determine compatibility. Clean-Blot IP Detection Reagent (HRP) might not detect mouse IgG₁.

2. Add the diluted primary antibody to the blot and incubate for 1 hour at room temperature or overnight at 4°C.
3. Wash blot with Wash Buffer.
4. Dilute the Clean-Blot IP Detection Reagent (HRP) with blocking buffer.

Note: See table above for dilution ranges. For best results, optimize the dilution for each specific experiment.

5. Add the diluted detection reagent to the blot and incubate for 1 hour at room temperature or overnight at 4°C
6. Wash blot with wash buffer.
7. Prepare and add the HRP substrate according to the manufacturer's instructions.
8. Expose to blot to film or CCD camera.

Troubleshooting

Problem	Possible Cause	Solution
Nonspecific bands	Too much HRP in the system	Dilute the Clean-Blot IP Detection Reagent
	Too much primary antibody	Dilute primary antibody
	SDS caused nonspecific binding to protein bands	Do not use SDS during immunoassay procedure
Speckled background on film	Aggregate formation in the Clean-Blot IP Detection Reagent (HRP)	Filter detection reagent through a 0.2 µm filter or centrifuge and use supernatant
Weak or no signal	Too much or not enough HRP in the system	Optimize the Clean-Blot IP Detection Reagent
	Insufficient quantities of antigen or antibody	Increase concentration of antibody or antigen
	Inefficient protein transfer	Optimize transfer
High background	Used too much detection reagent	Dilute the Clean-Blot IP Detection Reagent
	Inadequate blocking	Optimize blocking conditions
	Inappropriate blocking reagent	Try a different blocking reagent
	Inadequate washing	Increase length, number or volume of washes
	Film was overexposed	Decrease exposure time or use Pierce Background Eliminator (Product No. 21065)
	Antigen or antibody is too concentrated	Dilute the antigen or antibody

Problem	Possible Cause	Solution
Detection of denatured and blotted IgG	All IgG in the sample was not denatured	Increase reducing reagent concentration
		Boil sample to aid in reduction of IgG disulfide bonds
		Use denaturing electrophoresis conditions
Antigen of interest was not detected	Sample does not contain antigen or does not contain a detectable quantity of antigen	Optimize expression and lysis procedures
	Antibody does not recognize antigen	Use a different primary antibody
	Primary antibody is not compatible	Verify target by performing a dot blot using a traditional secondary antibody

Additional Information

Please visit the website for additional information on this product including the following:

- Tech Tip protocol: Optimization of antigen and antibody concentrations for Western blots
- Tech Tip protocol: Optimization of blocking buffer and cross-reactivity determination

Related Products

21233	Clean-Blot IP Detection Reagent (AP), 2.5 ml
34090	CL-Xposure™ Film, 5" × 7" sheets, 100 sheets/pkg
32106	Pierce ECL Western Blotting Substrate, 500 ml
34080	SuperSignal West Pico Chemiluminescent Substrate, 500 ml
34075	SuperSignal West Dura Extended Duration Substrate, 100 ml
34095	SuperSignal West Femto Maximum Sensitivity Substrate, 100 ml
21059	Restore™ Western Blot Stripping Buffer, 500 ml
21065	Pierce Background Eliminator Kit, for eliminating background from X-ray film
37515	SuperBlock (PBS) Blocking Buffer, 1 L
37535	SuperBlock (TBS) Blocking Buffer, 1 L
37542	StartingBlock (TBS) Blocking Buffer, 1 L
37538	StartingBlock (PBS) Blocking Buffer, 1 L
88018	Nitrocellulose Membrane, 0.45 µm, 33 cm × 3 m, 1 roll
77010	Nitrocellulose Membrane, 0.45 µm, 8 × 12 cm, 25/pkg.
88025	Nitrocellulose Membrane, 0.45 µm, 8 × 8 cm, 15/pkg.
88600	Western Blotting Filter Paper, 8 cm × 10.5 cm, 100 sheets
24580	MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes
24585	MemCode Reversible Protein Stain Kit for PVDF Membranes
28320	Surfact-Amps® 20, 6 × 10 ml ampules containing 10% solutions of Tween-20 Detergent
28374	BupH™ Modified Dulbecco's PBS Packs, 40 packs, each yielding 500 ml
28376	BupH Tris Buffered Saline Packs, 40 packs, each yielding 500 ml

SuperSignal[®] Technology is protected by U.S. Patent # 6,432,662
Tween[®] is a registered trademark of ICI Americas.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2008 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.