INSTRUCTIONS

Pierce™ Far-Western Biotinylated-Protein:Protein Interaction Kit

23500

1344.3

Number	Description
23500	**Pierce Far-Western Biotinylated-Protein:Protein Interaction Kit,** sufficient materials for 10 mini-gel or membrane (1000cm^2) detections using a biotinylated-protein probe

Kit Contents:

Streptavidin-Horseradish Peroxidase (Streptavidin-HRP), 1mg, lyophilized

Dilution Buffer (10X), 50mL

BupH™ Phosphate Buffered Saline Packs, 17 packs, 0.1M phosphate, 0.15M NaCl, pH 7.2

Luminol Enhancer, 55mL

Stable Peroxide, 55mL

Surfact-Amps™ 20 Detergent Solution, 6 × 10mL vials, 10% solution

Pre-Cut Cellophane, 10 sheets

Storage: Upon arrival store kit at 4°C. Kit is shipped at ambient temperature. The Streptavidin-HRP is stable for six months at 4°C after reconstitution.

Table of Contents

Introduction 2
Important Product Information 2
Material Preparation 3
Procedure for Membrane Far-Western Detection 3
Procedure for In-Gel Far-Western Detection 4
Procedure for Gel Staining with GelCode Blue Stain Reagent after In-Gel Detection 6
Troubleshooting 7
Additional Information 8
Related Thermo Scientific Products 8
References 8

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce

Introduction

Far-Western blotting is the method of probing a membrane containing transferred protein with another protein to detect specific protein-protein interactions.[1] The Thermo Scientific™ Pierce™ Far-Western Biotinylated-Protein:Protein Interaction Kit makes it possible to detect "prey" protein separated by SDS-PAGE with a biotinylated bait or "probe" protein and a streptavidin-HRP chemiluminescent detection system. This kit can be used for both on-membrane or in-gel discovery of protein:protein interactions. In-gel detection is possible with an optimized pretreatment step and an extremely sensitive substrate.[2]

In-gel detection has advantages over on-membrane detection for several reasons. For example, protein transfer efficiency depends on protein electroelution efficiency, gel pore size and the proteins' molecular weight and net charge. Transfer conditions suited for one protein may not be optimal for another because smaller proteins transfer more efficiently than larger proteins. The membrane pore size may also affect overall protein immobilization, and diffusion during electrotransfer may affect protein band width on the membrane.[3] Some proteins have been reported to bind selectively to membranes.

Furthermore, far-Western blotting methods require that the "probe" protein be tagged or have an antibody produced against it. Protein tags are often made by recombinant protein expression in cell-free systems. This type of a protein tagging method is time-consuming and not always practical. However, the biotin molecule is small and can be easily conjugated to proteins.

The Pierce Far-Western Biotinylated-Protein:Protein Interaction Kit overcomes the limitations of traditional protein:protein interaction discovery with an effective method to directly detect proteins in a polyacrylamide gel. Gels can be electrophoresed using native or denaturing conditions, and because there is no need to transfer proteins, results are obtained within four hours after gel electrophoresis. The kit is flexible, however, and also can be used for on-membrane detection if sample amount is limited or the protein:protein interaction is weak.

Important Product Information

- This product is designed for detecting antigens using purified biotinylated proteins.
- The following pre-cast gel brands and types work well with this Pierce In-Gel Detection Technique: Novex™, FMC, BioWhittaker and Bio-Rad Criterion™ brand Bis-Tris and Tris-Glycine gels (both native and denaturing).
- Pre-cast Bio-Rad, iGel and Zaxis gels do not perform well with UnBlot In-Gel Detection. Sensitivity with these gel types is 25X less, and they may require individual optimization. If one of these gel types is used, transfer proteins to a membrane and follow the procedure for membrane far-Western detection.
- Homemade gels have not been optimized for use with UnBlot In-Gel Detection.
- For optimal results use gels that are 0.75-1.5mm thick.
- Gradient gels of 3-8%, 4-12%, 8-18%, 4-20% and 10-20%, as well as homogeneous gels (8-16%) work well.
- Proteins of MW 20,000-160,000 have been detected successfully with UnBlot In-Gel Detection.
- Use a clean knife or plastic spatula to manipulate the gel. Do not touch gel with hands or background may result.
- Use a platform shaker for all incubation steps. Keep gel submerged during all incubation and wash steps.

Optimization of the biotinylated-protein probe and Streptavidin-HRP dilution is necessary to achieve the best results for specific applications.

Material Preparation

Note: Dilution and wash buffers are provided in the kit; however, these may not be optimal for the specific protein:protein interaction system being investigated, and other buffers may be substituted. If cofactors are required to promote the interaction of interest, then they may be added to the buffer.

Purified "prey" protein	Dilute purified protein to twice the desired concentration in a buffer such as PBS, then combine with an equal volume of 2X SDS-PAGE or 2X Native Tris-Glycine sample buffer. Prepare the final dilution so that 10µL of the diluted sample contains 1-10ng of protein. Heat for 5 minutes at 95°C. Eliminate the heating step if heating affects the specific protein interaction of interest.
Lysates	For "prey" protein in lysates, dilute lysate 1:2 to 1:100 in 2X SDS-PAGE or 2X Native Tris-Glycine sample buffer. Heat for 5 minutes at 95°C. Eliminate the heating step if heating affects the specific protein interaction of interest.
PBS with 0.05% Tween™-20 (PBS-T)	For a 2L preparation, use 4 packets of Thermo Scientific™ BupH™ Phosphate Buffered Saline and one ampoule of Surfact-Amps 20 Detergent Solution. The volume required per gel is approximately 650-700 ml. Reserve 1 ml of the PBS without the Tween-20 Detergent to prepare the Streptavidin-HRP.
Biotinylated-protein probe	Biotinylate the probe protein using standard procedures. Suggested biotinylation reagents are listed in the Additional Information section.
Streptavidin-HRP	Reconstitute the 1mg Streptavidin-HRP with 1mL of PBS.
Dilution Buffer	Add 4mL of Dilution Buffer (10X) to 36mL of PBS-Tween-20 (PBS-T).
Substrate Working Solution	Mix 5mL of Stable Peroxide with 5mL of Luminol Enhancer. Prepare Working Solution just before use. **Note:** Exposure to the sun or other intense light can harm the Working Solution. For best results, keep Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Typical laboratory lighting will not harm the Working Solution.
50% isopropyl alcohol	Add 25mL of 100% isopropyl alcohol (not provided) to 25mL of ultrapure water and mix. (Required for In-Gel Far-Western Detection only).

Procedure for Membrane Far-Western Detection

Notes:

- **Membrane vs. In-gel detection:** Although this kit is designed for detection of "prey" protein directly within a gel, it is also optimized for detection on membranes. If protein:protein interactions are weak or samples are only available in limited quantities, it may be necessary to transfer the "prey" proteins from the gel to a membrane and perform the detection on the membrane. The membrane system is 5-10 times more sensitive than the in-gel detection method with the substrate provided in this kit. Also, because this kit has been optimized for detection of "prey" protein both within the gel and on membrane, results obtained with the in-gel detection method may be confirmed on the membrane.
- **Negative control:** Test for the possible presence of endogenous biotin or nonspecific binding of Streptavidin-HRP to protein bands by performing the following steps:
  1. Perform SDS-PAGE or native gel electrophoresis on two identical gels, and transfer the contents to membranes. Use one membrane as a negative control.
  2. Follow with the Membrane Far-Western Procedure until step 5, at which point incubate the negative control membrane with the Dilution Buffer alone (i.e., no biotinylated protein probe) or with non-biotinylated probe while incubating the test membrane with the biotinylated protein probe according the instructions.
  3. Complete the Membrane Far-Western Procedure with both membranes.
- **Membrane handling:** Use a clean forceps to handle membranes. Do not touch membranes with hands or high background may result. Use a platform shaker for all of the incubation steps, and ensure that membranes are completely submerged throughout all incubation and wash steps. Use a second clean container for the substrate incubation step.
- **Cofactors and Binding/Wash Buffers:** Specific cofactors may be included in the dilution and wash buffer provided or in other buffers optimal for a particular application.

1. After electrophoresis, remove the top plate of gel cassette. Use a knife to cut off and discard the top (stacking) portion of gel, approximately 0.5-1cm from the bottom of the wells.
2. Transfer protein samples from gel to a membrane.
3. After the transfer step, block nonspecific sites with Dilution Buffer or an alternative blocking buffer. Block overnight at 4°C or for 1 hour at room temperature (RT).
4. Dilute biotinylated-protein probe in Dilution Buffer to a final concentration of 0.1-5µg/mL in 10mL. For example, to prepare 1µg/mL solution, add 10µL of a 1mg/mL biotinylated-protein probe stock to 10mL of Dilution Buffer.
5. Decant Dilution Buffer from container. Add diluted biotinylated-protein probe solution to the container and incubate membrane for 1 hour at RT. Membrane must float freely in the solution.
6. Wash membrane 6 × 5 minutes each with 50mL PBS-T. Membrane must float freely and be shaken gently.
7. Dilute the reconstituted 1 mg/ml Streptavidin-HRP 1:100,000-1:500,000 in Dilution Buffer. For example, to prepare a 1:100,000 dilution, first prepare a 1:100 dilution by adding 10µL of the reconstituted Streptavidin-HRP (1mg/mL) to 990µL of the Dilution Buffer in a microcentrifuge tube and mix. Then add 10µL of the 1:100 diluted Streptavidin-HRP to 9.99mL of Dilution Buffer.
8. Decant wash buffer and add diluted Streptavidin-HRP solution. Incubate membrane for 1 hour at RT.
9. Wash membrane 6 × 5 minutes each with 100mL PBS-T. Membrane must float freely and be shaken gently.
10. Prepare Substrate Working Solution (see Material Preparation Section).

    Note: Exposure to the sun or other intense light can harm the Working Solution. For best results keep Working Solution in an amber bottle and avoid prolonged exposure to intense light. Laboratory lighting will not harm Working Solution.
11. Add 10mL of Substrate Working Solution to a clean container.
12. Transfer the membrane to the container with the Substrate Working Solution. Incubate membrane in the Substrate Working Solution for 5 minutes at RT with gentle shaking.
13. Place membrane between two cellophane sheets. Expose the membrane "sandwich" to film for different lengths of time and develop the film, or expose membrane to a CCD camera. The chemiluminescent signal can be detected for up to 2 hours following the substrate incubation step.

    Note: Try exposing film for 1 minute. If no bands are detectable, then increase exposure time to 5 minutes or longer. If the 1 minute exposure produces overly dark bands and background, decrease exposure time to 5-30 seconds.

Procedure for In-Gel Far-Western Detection

Notes:

- **In-gel vs. membrane detection:** Although this kit is designed for detection of "prey" protein directly within a gel, it is also optimized for detection on membranes. If protein:protein interactions are weak or samples are only available in limited quantities, it may be necessary to transfer the "prey" proteins from the gel to a membrane and perform the detection on the membrane. The membrane system is 5-10 times more sensitive than the in-gel detection method with the substrate provided in this kit. Also, because this kit has been optimized for detection of "prey" protein both within the gel and on membrane, results obtained with the in-gel detection method may be confirmed on the membrane.
- **Negative control:** Test for the possible presence of endogenous biotin or nonspecific binding of Streptavidin-HRP to protein bands by performing the following steps:

    1. Perform SDS-PAGE or native gel electrophoresis on two identical gels and use one gel as a negative control.
    2. Follow with the Procedure for In-Gel Detection until step 6, at which point incubate the negative control gel with the Dilution Buffer alone (i.e., no biotinylated protein probe) or with non-biotinylated probe while incubating the test gel with the biotinylated protein probe according the instructions.
    3. Complete the Procedure for In-Gel Detection with both gels.

- **Gel handling:** Use a clean knife or plastic spatula to manipulate the gel. Do not touch the gel with hands or high background may result. Use a platform shaker for all of the incubation steps. The gel must be completely submerged throughout all incubation and wash steps. Use the Incubation Colander (Product No. 33499, see Additional Information section) or other suitable container for the incubation and wash steps. Use a second clean container for the substrate incubation step.
- **Cofactors and Binding/Wash Buffers:** Specific cofactors may be included in the dilution and wash buffer provided or in other buffers optimal for a particular application.

1. After electrophoresis, remove the top plate of gel cassette. Use a knife to cut off and discard the top (stacking) portion of gel, approximately 0.5-1cm from the bottom of the wells (see Figure 1).

   Note: Removing the top of gel reduces background during film exposure. If the protein of interest is of high M.W., cut only a small amount from the top of the gel, or remove this part of the gel after the first exposure if it causes high background.

   Note: Notching one top corner of gel (see Figure 1) can assist in orientating the results when detectable molecular weight markers are not used.

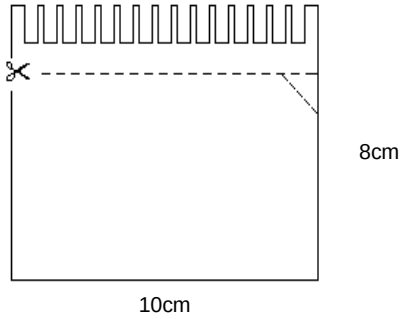

Figure 1: Gel cutting and orientation.

2. Carefully transfer gel to or Hands-Off Incubation Colander (see Related Thermo Scientific Products) or other suitable container.
3. Pretreat gel by adding 50mL of 50% isopropyl alcohol. Gently shake gel in this solution for 15 minutes.
4. Replace alcohol with 100mL of ultrapure water. Wash gel with gentle shaking for 15 minutes.

   Note: Between steps, completely drain all liquid from the container. Remove excess liquid from the container by tapping and/or blotting on a clean paper towel. Do not allow the gel to touch the paper towel.

   Note: During the wash step, prevent gel curling by gently flattening the gel with a gel knife or spatula or flipping the gel over. The gel must be completely submerged in water to remove excess alcohol from the gel surface.

   This can be a stopping point. Gel can be left in ultrapure water or 10% isopropyl alcohol overnight at 4°C.

5. Dilute the biotinylated-protein probe to a final concentration of 1-20µg/mL in 20mL of Dilution Buffer. For example, to prepare 1µg/mL solution, add 20µL of a 1mg/mL biotinylated-protein probe stock to 20mL of Dilution Buffer.

   Note: See the Additional Information section for suggested reagents for biotinylating protein probes.

   Note: If the biotinylated-protein probe is in limited quantity, prepare only 10mL of the diluted biotinylated-protein probe and perform the incubation step in a smaller container (e.g., a petri dish). Unused portions of the gel can be excised so that it fits in the smaller container. All wash steps can be performed in a regular container.
6. Decant water from gel container. Add diluted biotinylated-protein probe solution to the container and incubate gel for 1 hour at room temperature (RT). Make sure gel floats freely in the solution.
7. Wash gel 3 × 10 minutes each with 100mL of PBS-T. Gel must float freely in the solution and be shaken gently.
8. Dilute the reconstituted 1mg/mL Streptavidin-HRP 1:10,000-1:50,000 in Dilution Buffer. For example, to prepare 1:10,000 dilution, first prepare a 1:10 dilution by adding 5µL of the reconstituted Streptavidin-HRP (1mg/mL stock) to 45µL of Dilution Buffer in a microcentrifuge tube and mix. Then add 10µL of the 1:10 diluted Streptavidin-HRP to 10mL of Dilution Buffer.
9. Decant wash buffer and add diluted Streptavidin-HRP solution. Incubate gel for 1 hour at RT.

10. Wash gel 3 × 10 minutes each with 100mL of PBS-T. Gel must float freely in the solution and be shaken gently.
11. Prepare Substrate Working Solution (see Material Preparation Section).

    Note: Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Typical laboratory lighting will not harm the Working Solution.
12. Add 10mL of Substrate Working Solution to a clean container.
13. Transfer the gel to the container with the Substrate Working Solution.

    Note: If using a colander device, use only the bottom tray for substrate development.
14. Incubate gel in 10mL of the Substrate Working Solution for 5 minutes at room temperature with gentle shaking. Both sides of gel must be exposed to the substrate.
15. Decant Substrate Working Solution. Add 50mL of ultrapure water, wash for 15 seconds and decant water.

    Note: Do not wash for more than 15 seconds or poor signal will result.
16. Place gel between two cellophane sheets. Remove excess liquid from around gel by wicking it away with a strip of filter paper or paper towel; do not allow paper to contact the gel surface. Gently smooth out air bubbles from gel "sandwich."
17. Expose the gel "sandwich" to film for different lengths of time and develop the film, or expose gel to a CCD camera. The chemiluminescent signal can be detected for up to 2 hours following the substrate incubation step.

    Note: Try exposing film for 1 minute. If no bands are detectable, then increase exposure time to 5 minutes or longer. If the 1-minute exposure produces overly dark bands and background, decrease exposure time to 5-30 seconds.

Procedure for Gel Staining with GelCode™ Blue Stain Reagent after In-Gel Detection

Note: Gels that have been through the Pierce In-Gel Detection procedure may require a destaining step when using Thermo Scientific™ GelCode™ Blue Stain Reagent (Product No. 24590).

1. Remove gel from cellophane and wash 3 × 5 minutes each with 50mL of ultrapure water. Leave gel immersed in the water wash for at least one hour or preferably overnight.

   Note: Insufficient washing will cause high background mainly at the top of the gel.
2. Incubate gel in 50mL of GelCode Blue Stain Reagent for 1 hour at room temperature.
3. Rinse gel 2 × 5 minutes each with 50mL of ultrapure water.
4. Destain gel with a 5% acetic acid/40% methanol solution. Replace destaining solution until gel and solution are clear.

   Note: This destaining step is required only if the background needs to be decreased quickly.
5. Place gel in water until ready for preservation.

Troubleshooting

Problem	Cause	Solution
High background at the top of gel	Not enough cut off from the top of the gel	Cut 0.5-1cm from the bottom of the comb
High background	Biotinylated-protein probe concentration is too high	Further dilute biotinylated-protein probe 2- to 5-fold
	Streptavidin-HRP concentration is too high (e.g., 1:10,000 dilution or 100ng/mL)	Reduce Streptavidin-HRP concentration (e.g., dilute 1:50,000 or 20ng/mL)
	Inadequate washes or gel was sticking to the bottom of the tray	Increase length (e.g., 3 × 20 min, 50mL/wash) or frequency (e.g., 6 × 5 min, 50mL/wash) of washes and make sure gel floats freely in solution during wash step
	UnBlot Substrate incubation too long and/or water wash was omitted	Reduce the substrate incubation time and/or follow with a water rinse
	Film exposure too long	Reduce film exposure (e.g., from 1 minute to 30 seconds or 15 seconds) or use Thermo Scientific™ Pierce™ Background Eliminator (Product No. 21065)
Low signal	Streptavidin-HRP is too dilute	Try 2- to 5-fold higher concentration
	Biotinylated-protein probe is too dilute	Try 2- to 5-fold higher biotinylated-protein probe concentration. Note: Alter the biotinylated "probe" (bait) protein concentration only if adjusting the Streptavidin-HRP concentration has no effect or results in high background.
	Probe protein not sufficiently biotinylated	Biotinylate probe (bait) protein again with a higher molar excess of the biotinylation reagent to the protein concentration; mole-to-mole ratio of biotin may be determined using HABA (Product No. 28010) each time the probe is biotinylated to make sure that consistent biotinylation level is obtained
	Probe protein excessively biotinylated and interfering with the protein:protein interaction	Biotinylate probe protein again with a lower molar excess of the biotinylation reagent to the protein concentration or try a biotinylation reagent that couples to a different functional group
	Biotinylated protein probe interacts weakly with "prey" protein	Use native conditions to separate samples, as denaturing conditions may alter the proteins such that they do not interact with the "probe"
Diffuse bands	Residual isopropyl alcohol remaining on gel surface following pretreatment step	Make sure gel does not stick to the bottom of the tray and/or is totally submerged and freely floating during water wash
	Gel was running too fast	Decrease voltage during electrophoresis
	Expired gels	Do not use gels that have expired. Expired gels affect in-gel detection more significantly than on-membrane detection
Fuzzy bands	Protein degradation	Prepare new protein samples
Nonspecific bands	Incompatible Dilution Buffer	Use a compatible blocking buffer to dilute the biotinylated-protein probe and Streptavidin-HRP
No bands	Protein did not migrate from stacker; % gel was too high	Use lower % gel
	Biotinylated-protein probe was not recognizing "prey" protein	Select a biotinylation reagent that couples to a different functional group or biotinylate with a lower molar excess of the biotinylation reagent to the protein concentration
	"Bait" protein denatured by SDS-PAGE	Use native conditions to separate samples, as denaturing conditions may alter the proteins such that they do not interact with the "probe"

Additional Information

A. To biotinylate antibodies in the laboratory, use one of the following reagents:

- Sulfo-NHS-LC-Biotin (Product No. 21335): This reagent biotinylates the IgG through the primary amine groups on the antibody and is most commonly used to biotinylate antibodies. The procedure for biotinylation is simple and can be performed while the gel is being electrophoresed. This reagent is also available in a kit format (Product No. 21420).
- Biotin-BMCC (Product No. 21900): This reagent biotinylates the IgG through the sulfhydryl groups that are produced by reduction of the antibody with 2-Mercaptoethylamine•HCl (Product No. 20408) or introduced through the primary amine groups of the antibody by *N*-Succinimidyl-S-acetylthioacetate (SATA, Product No. 26102) or 2-Iminothiolane•HCl (Traut's Reagent, Product No. 26101)
- Biotin-LC-Hydrazide (Product No. 21340): This reagent biotinylates the IgG through the carbohydrate moieties on the Fc region.

B. The Incubation Colander device

Using the gel colander device (Product No. 33499) for gel incubation and wash steps reduces gel handling and, therefore, results in less chance of gel tearing. The device is supplied with two bottom trays and one colander (Figure 2).

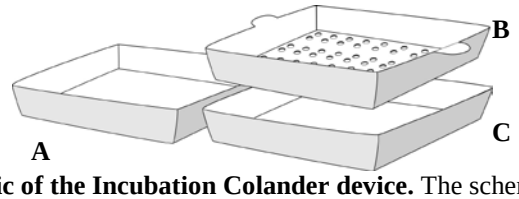

Figure 2. Schematic of the Incubation Colander device. The schematic is labeled as follows: A and C are the bottom trays and B is the colander.

Related Thermo Scientific Products

33500	**Pierce In-Gel Chemiluminescent Detection Kit—Rabbit**
33505	**Pierce In-Gel Chemiluminescent Detection Kit—Mouse**
33550	**Pierce In-Gel Chemiluminescent Substrate**, 110mL
34090	**CL-XPosure Film**, 5" × 7" sheets, 100 sheets/pkg
21059	**Restore™ Western Blot Stripping Buffer**, 500mL
21065	**Pierce Background Eliminator**, 200mL
24590	**GelCode Blue Stain Reagent**, 500mL

Cited References

1. Burgess, R., Arthur, T.M. and Pietz, B.C. (2000). Mapping protein-protein interaction domains using ordered fragment ladder far-Western analysis of hexahistidine-tagged fusion proteins. *Meth Enzymol* **328:**141-57.
2. Desai, S., Dworecki, B. and Cichon, E. (2001). Direct immunodetection of antigens within precast polyacrylamide gels. *Anal Biochem* **297:**84-98.
3. Lin, W. and Kasamatsu, H. (1983). On electrotransfer of polypeptides from gels to nitrocellulose membranes. *Anal Biochem* **128:**302-11.
4. Reddy, V.M. and Kumar, B. (2000). Interactions of *Mycobacterium avium* complex with human respiratory epithelial cells. *JID* **181:**1189-93.

Products are 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"). 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 Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.

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 REPAIR, REPLACEMENT OF OR REFUND FOR THE NON-CONFORMING PRODUCT(S) AT SELLER'S SOLE OPTION. THERE IS NO OBLIGATION TO REPAIR, REPLACE OR REFUND FOR 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.

Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals.

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

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermoscientific.com/pierce