## Pierce™ Kinase Enrichment Kits and ActivX™ Probes

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>88310</td>
<td>Pierce Kinase Enrichment Kit with ATP Probe, contains sufficient reagents for 16 pull-downs</td>
</tr>
<tr>
<td></td>
<td>Kit Contents:</td>
</tr>
<tr>
<td></td>
<td>ActivX Desthiobiotin-ATP Probe, 16 × 12.6μg</td>
</tr>
<tr>
<td></td>
<td>Pierce IP Lysis Buffer, 100mL</td>
</tr>
<tr>
<td></td>
<td>Reaction Buffer, 125mL</td>
</tr>
<tr>
<td></td>
<td>Halt™ Protease/Phosphatase Inhibitor Cocktail (100X), 1mL</td>
</tr>
<tr>
<td></td>
<td>Zeba™ Spin Desalting Columns, 7K MWCO, 5mL, 8 each</td>
</tr>
<tr>
<td></td>
<td>High Capacity Streptavidin Agarose Resin (50% slurry), 1mL</td>
</tr>
<tr>
<td></td>
<td>MgCl₂ (1M), 500μL</td>
</tr>
<tr>
<td></td>
<td>Urea, 12g</td>
</tr>
<tr>
<td></td>
<td><strong>Storage:</strong> ActivX Desthiobiotin-ATP Probe is shipped separately with dry ice and stored at -80°C upon receipt. All other kit components are shipped at ambient temperature and stored at 4°C upon receipt.</td>
</tr>
<tr>
<td>88311</td>
<td>ActivX Desthiobiotin-ATP Probe, 16 × 12.6μg</td>
</tr>
<tr>
<td></td>
<td><strong>Molecular Weight:</strong> 1259.48</td>
</tr>
<tr>
<td></td>
<td><strong>Storage:</strong> Upon receipt store at -80°C. Product is shipped with dry ice.</td>
</tr>
<tr>
<td>88312</td>
<td>Pierce Kinase Enrichment Kit with ADP Probe, contains sufficient reagents for 16 pull-downs</td>
</tr>
<tr>
<td></td>
<td>Kit Contents:</td>
</tr>
<tr>
<td></td>
<td>ActivX Desthiobiotin-ADP Probe, 16 × 9.9μg</td>
</tr>
<tr>
<td></td>
<td>Pierce IP Lysis Buffer, 100mL</td>
</tr>
<tr>
<td></td>
<td>Reaction Buffer, 125mL</td>
</tr>
<tr>
<td></td>
<td>Halt Protease/Phosphatase Inhibitor Cocktail (100X), 1mL</td>
</tr>
<tr>
<td></td>
<td>Zeba Spin Desalting Columns, 7K MWCO, 5mL, 8 each</td>
</tr>
<tr>
<td></td>
<td>High Capacity Streptavidin Agarose (50% slurry), 1mL</td>
</tr>
<tr>
<td></td>
<td>MgCl₂ (1M), 500μL</td>
</tr>
<tr>
<td></td>
<td>Urea, 12g</td>
</tr>
<tr>
<td></td>
<td><strong>Storage:</strong> ActivX Desthiobiotin-ADP Probe is shipped separately with dry ice and stored at -80°C upon receipt. All other kit components are shipped at ambient temperature and stored at 4°C upon receipt.</td>
</tr>
<tr>
<td>88313</td>
<td>ActivX Desthiobiotin-ADP Probe, 16 × 9.9μg</td>
</tr>
<tr>
<td></td>
<td><strong>Molecular Weight:</strong> 994.15</td>
</tr>
<tr>
<td></td>
<td><strong>Storage:</strong> Upon receipt store at -80°C. Product is shipped with dry ice.</td>
</tr>
</tbody>
</table>
Introduction

The Thermo Scientific™ Pierce™ Kinase Enrichment Kits with ActivX ATP and ADP Probes enable selective labeling and enrichment of ATPases including kinases, chaperones and metabolic enzymes. Thermo Scientific™ ActivX™ Desthiobiotin-ATP and -ADP Probes are nucleotide derivatives, which covalently modify the active site of enzymes with conserved lysine residues in the nucleotide-binding site. The structure of desthiobiotin-ATP and -ADP consists of a modified biotin attached to the nucleotide by a labile acyl-phosphate bond (Figure 1). Depending on the position of the lysine within the enzyme active site, either desthiobiotin-ATP or -ADP may be better for labeling specific ATPases.

Both desthiobiotin-ATP and -ADP can selectively enrich, identify and profile target enzyme classes in samples or assess the specificity and affinity of enzyme inhibitors. Many ATPases and other nucleotide-binding proteins bind nucleotides or inhibitors even when they are enzymatically inactive; these reagents bind both inactive and active enzymes in a complex sample. Preincubation of samples with small-molecule inhibitors that compete for active-site probes can be used to determine inhibitor binding affinity and target specificity.

Assessment of active-site labeling can be accomplished by either Western blot or mass spectrometry (MS) (Figure 2). For the Western blot workflow, desthiobiotin-labeled proteins are enriched for SDS-PAGE analysis and subsequent detection with specific antibodies. For the MS workflow, desthiobiotin-labeled proteins are reduced, alkylated and enzymatically digested to peptides. Only the desthiobiotin-labeled, active-site peptides are enriched for analysis by LC-MS/MS. Both workflows can be used for determining inhibitor target binding, but only the MS workflow can identify global inhibitor targets and off-targets.

![Figure 1. Chemical structures of the Thermo Scientific ActivX Desthiobiotin-ATP and -ADP Probes.](image)

![Figure 2. Western blot and mass spectrometry workflows enable targeted capture and analysis of enzymes using the active-site probes.](image)
Important Product Information

- Desthiobiotin-ATP and -ADP are supplied in the Thermo Scientific™ No-Weigh™ Format, which enables single-use preparations. Note that the product is typically not visible in the vial.

- Desthiobiotin-ATP and -ADP are moisture-sensitive. To avoid moisture condensation onto the product, equilibrate vial to room temperature before opening. Prepare the labeling reagents immediately before use. The acyl-phosphate linkage readily hydrolyzes and becomes non-reactive; therefore, do not prepare stock solutions for storage. Discard any unused reconstituted compound.

- Desthiobiotin-ATP and -ADP are temperature-sensitive. Minimize exposure of product to ambient temperatures by returning unused product to -80°C between uses.

- Desthiobiotin-ATP and -ADP typically label accessible enzyme active sites, regardless of activity; however, some active enzymes might be preferentially labeled.

- The Pierce IP Lysis Buffer is effective for lysing cultured mammalian cells from both plated cells and cells pelleted from suspension cultures. Sonication is not required but might be necessary to fully lyse some cell types. For tissues, perform mechanical homogenization.

- Desalting of lysates using the Thermo Scientific™ Zeba™ Spin Desalting Columns or equivalent is required to remove endogenous ATP for optimal labeling.

- Adding MgCl₂ is required for labeling. MnCl₂ may be substituted for MgCl₂ in labeling reactions for active-site peptide labeling; however, proteins might precipitate.

- Adding urea to samples after labeling is required to denature proteins before capture using streptavidin agarose. Make urea buffers the same day as the experiment.

- For best results, perform labeling reactions using 5μM of desthiobiotin-ATP or -ADP when assessing inhibitor binding. Add up to 20μM for maximal protein enrichment. Labeling reactions using concentrations > 20μM require additional desalting to remove non-reacted probe before streptavidin capture.

- Desthiobiotin modification of lysine-containing active-site peptides results in a monoisotopic mass increase of 196.1212 Da.

Procedure for Protein Labeling and Enrichment

Note: This protocol is for labeling 2 × 1mg samples with 5-20μM of probe. Scale the procedure accordingly for other amounts.

A. Additional Materials Required

- Ice-cold phosphate-buffered saline (PBS): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (Product No. 28372)
- Protein Assay: Thermo Scientific™ Pierce™ BCA Protein Assay Reagent Kit (Product No. 23224) or Pierce™ 660nm Protein Assay Reagent (Product No. 22660)
- Scissors
- Variable-speed centrifuge
- 15mL conical collection tubes or equivalent
- Rotary mixer
- Microcentrifuge
- 1.7mL microcentrifuge tubes or equivalent
- 2X Laemmli reducing sample buffer (Product No. 84788 or equivalent)
- Optional: 1M MnCl₂
B. Material Preparation

- **8M Urea/IP Lysis Buffer**: Dissolve 0.75g of urea with 1.5mL of IP Lysis Buffer for each labeling reaction.
- **4M Urea/IP Lysis Buffer**: Dilute 1mL of 8M Urea/IP Lysis Buffer with 1mL of Pierce IP Lysis Buffer for each labeling reaction.

C. Cell Lysis

1. For adherent cells, harvest with trypsin-EDTA and then centrifuge at 500 × g for 5 minutes. For suspension cells, harvest by centrifuging at 500 × g for 5 minutes. For tissues, cut 50-100mg of tissue into small pieces.
2. Wash cells by suspending the cell pellet with ice-cold PBS.
3. Transfer 2-4 × 10⁷ cells to a 1.5mL microcentrifuge tube. Pellet cells by centrifugation at 500 × g for 2-3 minutes and remove the PBS.
4. Add 1mL of Pierce IP Lysis Buffer containing protease and phosphatase inhibitors (1:100) and incubate on ice for 10 minutes with periodic mixing. Homogenize tissue using a Dounce homogenizer or tissue grinder.
5. Centrifuge tube at 16,000 × g at 4°C for 5 minutes.
6. Transfer the supernatant (total lysate) to a new tube.

D. Lysate Buffer-exchange

1. Twist off the Zeba Spin Desalting Column’s bottom closure and loosen cap. Place column in a 15mL collection tube.
2. Centrifuge column at 1000 × g for 2 minutes at room temperature to remove storage solution.
   - **Note**: Resin will appear compacted and dry after centrifugation.
3. Add 3mL of Reaction Buffer to the column. Centrifuge at 1000 × g for 2 minute to remove buffer. Repeat this step two additional times, discarding buffer from the collection tube.
   - **Note**: If buffer is not completely removed after final spin, centrifuge 1000 × g for an additional 2-3 minutes.
4. Place column in a new collection tube and slowly apply 1mL of lysate to the center of the compact resin bed.
5. Centrifuge at 1000 × g for 2 minutes to collect the sample. Discard column after use.
6. Add more protease/phosphatase inhibitor cocktail to sample (1:100) and place on ice until labeling (Section E).
   - **Note**: The samples may be snap-frozen with liquid nitrogen and stored at -80°C.

E. Sample Labeling

1. Perform a protein assay to measure the lysate’s protein concentration.
2. Dilute lysate with Reaction Buffer to 2mg/mL and transfer 500µL (1mg) to a microcentrifuge tube.
3. Add 10µL of 1M MgCl₂ to each sample, mix and incubate for 1 minute at room temperature.
   - **Note**: For best results, use MnCl₂ for active-site peptide labeling using the MS workflow.
4. If profiling an ATPase active-site inhibitor, add inhibitor to sample, mix and incubate 10 minutes at room temperature.
5. Equilibrate desthiobiotin-ATP or -ADP reagent to room temperature in pouch with desiccant.
6. Use scissors to cut off the single-use tubes needed and immediately return unused tubes to -80°C.
7. For a 5μM reaction (see Important Product Information), reconstitute reagent by adding 40µL of ultrapure water to make a 0.25mM stock solution.
   - **Note**: For 20μM reaction, reconstitute reagent by adding 10µL of ultrapure water to make a 1mM stock solution.
8. Add 10µL of desthiobiotin-ATP or -ADP stock to each sample and incubate for 10 minutes at room temperature.
F. Labeled Protein Capture and Elution
1. Add 500μL of 8M Urea/IP Lysis Buffer to each reaction for a total volume of 1mL.
2. Add 50μL of 50% High Capacity Streptavidin Agarose resin slurry to each sample and incubate for 1 hour at room temperature with constant mixing on a rotator.
   **Note:** Removal of the agarose storage buffer is not necessary. Mix agarose thoroughly and use a wide-bore pipette tip to transfer equal amounts of resin to each sample.
3. Centrifuge samples at 1000 × g for 1 minute to pellet resin. Remove supernatant.
4. Add 500μL of 4M Urea/IP Lysis Buffer and vortex briefly to mix. Centrifuge samples at 1000 × g for 1 minute to pellet resin. Repeat this step two additional times, discarding buffer after each wash.
5. Elute bound proteins by adding 2X Laemmli reducing sample buffer and boiling for 5 minutes.
6. Analyze eluted proteins by SDS-PAGE and Western blot.

Procedure for Active-Site Peptide Enrichment
**Note:** This protocol is a method to generate and enrich active site-labeled peptides for MS analysis. Perform Sections C-E from Procedure for Protein Labeling and Enrichment and then proceed with the following protocol.

A. Additional Materials Required
- Zeba Spin Desalting Columns, 7K MWCO, 5mL (Product No. 89891)
- 1M Tris-HCl, pH 8.0
- Phosphate-buffered saline (PBS): 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (Product No. 28372)
- Trypsin endoproteinase, modified, TPCK-treated, MS-Grade (Product No. 90055)
- DTT, No-Weigh Format (Product No. 20291)
- Iodoacetamide, Single-Use (Product No. 90034)
- LCMS-grade acetonitrile (ACN, Product No. 51101)
- LCMS-grade water (Product No. 51140)
- Trifluoroacetic acid (TFA, Product No. 28904)
- Optional: Thermo Scientific™ Pierce™ Spin Columns (Product No. 69705)

B. Material Preparation
- 10M Urea/IP Lysis Buffer
  - Dissolve 0.9g of urea with 1.5mL of Pierce IP Lysis Buffer for each labeling reaction.
- Digestion Buffer (2M Urea/20mM Tris, pH 8.0)
  - Dissolve 2.4g of urea with 0.4mL 1M Tris, pH 8.0 and 19.6mL of LCMS-grade water.
- 500mM DTT
  - Dissolve 7.7mg of DTT with 0.1mL water.
- 1M Iodoacetamide
  - Dissolve 18.4mg of iodoacetamide with 0.1mL water.
- Elution Buffer (50% ACN, 0.1% TFA)
  - Dilute 10μL of TFA with 5mL of ACN and 5mL of LCMS-grade water.
- 0.1% TFA
  - Dilute 10μL of TFA with 10mL of LCMS-grade water.

C. Labeled Protein Reduction and Alkylation
1. Add 500μL of 10M Urea/IP Lysis Buffer to each reaction for a total volume of 1mL.
2. Add 10μL of 500mM DTT to each sample and incubate at 65°C for 30 minutes.
3. Cool samples to room temperature, add 40μL of 1M iodoacetamide to each sample and incubate for 30 minutes protected from light.
D. Buffer Exchange
1. Twist off the Zeba Spin Desalting Column’s bottom closure and loosen cap. Place column in a 15mL collection tube.
2. Centrifuge column at 1000 × g for 2 minutes at room temperature to remove storage solution.
   **Note:** Resin will appear compacted and dry after centrifugation.
3. Add 3mL of Digestion Buffer to the column. Centrifuge at 1000 × g for 2 minute to remove buffer. Repeat this step two additional times, discarding buffer from the collection tube.
   **Note:** If buffer is not completely removed after final spin, centrifuge at 1000 × g for an additional 2-3 minutes.
4. Place column in a new collection tube and slowly apply 0.5mL of each reaction to the center of the compact resin bed.
5. Centrifuge at 1000 × g for 2 minutes to collect the sample. Discard column after use.

E. Labeled Protein Digestion
1. Transfer desalted proteins to a new microcentrifuge tube.
2. Reconstitute the 20μg of MS-grade trypsin (1 vial) with 10μL of LCMS-grade water.
3. Add trypsin to sample and incubate at 37°C with shaking for 2 hours.

F. Labeled Peptide Capture and Elution
1. Add 50μL of 50% High Capacity Streptavidin Agarose resin slurry to each digested sample and incubate for 1 hour at room temperature with constant mixing on a rotator.
   **Note:** For all subsequent steps, vortex briefly after adding buffer, centrifuge samples at 1000 × g for 1 minute to pellet resin and discard supernatant. Washing resin may be facilitated by transferring resin to an optional Pierce Spin Column.
2. Wash resin three times with 500μL of Pierce IP Lysis Buffer.
3. Wash resin four times with 500μL of PBS.
4. Wash resin four times with 500μL of LCMS-grade water.
5. Elute peptides by adding 75μL of Elution Buffer and incubating sample for 3 minutes. Transfer the eluate to a new microcentrifuge. Repeat this step two additional times.
6. Pool eluate fractions and freeze before lyophilizing.
7. Lyophilize the samples in a vacuum concentrator. Store lyophilized samples at -20°C.
8. Resuspend the samples in 25μL of 0.1% TFA and inject 1-5μL directly onto an LC-MS/MS system (e.g., Thermo Scientific™ LTQ or LTQ Orbitrap™ XL Mass Spectrometer) for analysis.
### Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No or low amount of kinase (ATPase) captured</td>
<td>Insufficient amount of probe was used</td>
<td>Increase probe concentration to 20μM</td>
</tr>
<tr>
<td></td>
<td>Probe was degraded</td>
<td>Store probes at -80°C and minimize exposure to moisture and elevated temperatures</td>
</tr>
<tr>
<td></td>
<td>The optimal probe was not used</td>
<td>Specific kinases (ATPases) may label better with the ATP or ADP probe</td>
</tr>
<tr>
<td></td>
<td>Insufficient lysate was used</td>
<td>Increase protein amount &gt; 2mg/mL in labeling reaction</td>
</tr>
<tr>
<td></td>
<td>Lysis was incomplete</td>
<td>Sonicate lysate or add additional non-denaturing detergents</td>
</tr>
<tr>
<td></td>
<td>Lysate was not desalted</td>
<td>Desalt lysate to remove endogenous ATP</td>
</tr>
<tr>
<td></td>
<td>MgCl₂ or probe was not added</td>
<td>Add MgCl₂ or probe to labeling reactions</td>
</tr>
<tr>
<td></td>
<td>Proteins were not fully denatured after labeling</td>
<td>Increase urea final concentration to 6M before streptavidin enrichment</td>
</tr>
<tr>
<td>No inhibition of kinase (ATPase) when inhibitor was used</td>
<td>Too much probe was used</td>
<td>Decrease probe concentration to 5μM</td>
</tr>
<tr>
<td></td>
<td>Insufficient inhibitor was used</td>
<td>Increase inhibitor concentration</td>
</tr>
<tr>
<td></td>
<td>Inhibitor was added after probe</td>
<td>Pretreat lysates with inhibitors before probe labeling</td>
</tr>
<tr>
<td></td>
<td>Inhibitor does not bind active site</td>
<td>Use active-site inhibitors</td>
</tr>
<tr>
<td>No or low amount of active-site peptides captured</td>
<td>Protein digestion was incomplete</td>
<td>Increase trypsin amount and digestion incubation</td>
</tr>
<tr>
<td></td>
<td>Peptides were lost during sample handling</td>
<td>Use low protein binding tubes for lyophilization</td>
</tr>
</tbody>
</table>

### Related Thermo Scientific Products

- **20357**  High Capacity Streptavidin Agarose Resin, 2mL
- **78440**  Halt Protease and Phosphatase Inhibitor Cocktail (100X), 1mL
- **87787**  Pierce IP Lysis Buffer, 100mL
- **89891**  Zeba Spin Desalting Columns, 7K MWCO, 5mL, 5 ea

### Cited References


Desethylbiotin-ATP and -ADP probes are licensed exclusively from ActivX Biosciences, Inc. for research use only. ActivX Biosciences and Kinativ are trademarks of ActivX Biosciences. Additional information on ActivX and KiNativ assay services is available at [www.kinativ.com](http://www.kinativ.com).

### Limited Use Label License: ActivX ATP and ADP Probes and Kinase Enrichment Kits containing probe (Thermo Scientific Product # 88310, 88311, 88312, 88313)

Buyer may not sell, transfer or otherwise provide access to (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator is provided with a copy of this Limited Use Label License and agrees to be bound by its terms.

Commercial Purposes means any activity by a party for monetary or other consideration and includes, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes—including the search for new drugs, or new discovery methods and to research, develop, or derive products; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

Recipient agrees not to reformulate, alter or make any changes to the Probe without prior written permission from ActivX Biosciences, Inc.

If the buyer is not willing to accept the limitations of this limited use statement, Pierce Biotechnology, Inc. is willing to accept return of the unused product with a full refund. By opening and using this product, the buyer agrees to be bound by the conditions of this Limited Use Label License.

Notwithstanding the restrictions set forth herein, buyer may use the product(s) for any Commercial Purpose with a valid license from ActivX Biosciences, Inc. For information on acquiring a license to this product for purposes other than those set forth herein, contact proteomics.licensing@thermofisher.com, Pierce Biotechnology, Inc., 3747 North Meridian Road, Rockford, IL 61101 or ActivX Biosciences, Inc. (info@activx.com).

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.

© 2013 Thermo Fisher Scientific Inc. All rights reserved. Triton is a trademark of The Dow Chemical Company. All (other) trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.