Thermo Scientific Automated Solutions for Proteomic Workflows

Outside of the United States, please fax back to 815-968-7316.

This card for mailing in the U.S. only.

Areas of Interest *(Please specify all that apply)*
- ☐ Cell Lysis
- ☐ Desalting
- ☐ Crosslinkers
- ☐ Protease Assay
- ☐ Protein Detection
- ☐ Protein Interaction

How do you currently prepare samples?
- ☐ Manual
- ☐ Automated (please specify the name of the company/model) _____

How do you isolate/purify your target protein?
- ☐ Agarose / Sepharose
- ☐ Affinity Supports
- ☐ Sample Prep
- ☐ Multiplex Protein Profiling
- ☐ IMAC
- ☐ Magnetic Affinity Purification

Do you have a KingFisher instrument in your lab?
- ☐ No
- ☐ Yes

If yes, please specify the model:
- ☐ KingFisher
- ☐ KingFisher mL
- ☐ KingFisher 96
- ☐ KingFisher Flex

How many samples do you analyze (quantities) per week?
- ☐ 1-30 samples/week
- ☐ 31-60 samples/week
- ☐ 61-90 samples/week
- ☐ 91-120 samples/week

Which downstream applications would you perform with isolated/purified protein(s) and the magnetic beads?
- ☐ Immunoprecipitation
- ☐ Therapeutic protein purification
- ☐ Phosphopeptide enrichment
- ☐ Western Blot Analysis
- ☐ SDS-PAGE
- ☐ MS analysis
- ☐ Other (Please specify) _____

Name _____ Title _____

Institution _____ Bldg./Rm. # _____

Address _____

City _____ State _____ Zip Code _____

Country _____

Phone ( ) _____ Fax ( ) _____

E-mail * _____

☐ Please have a Proteomics Applications Specialist contact me to discuss Automated Solutions. I can be reached at:

Phone ( ) _____ email * _____

*By providing my email address, I acknowledge that I may occasionally receive new product announcements from the Thermo Fisher Scientific Inc. Pierce Protein Research Product Line via email.

For immediate information: Internet: www.thermo.com/pierce • Tel: 800-874-3723 or 815-968-0747 • Fax: 815-968-7316

1601696 08/08 AUTO

Thermo Scientific Automated Solutions for Proteomic Workflows

Outside of the United States, please fax back to 815-968-7316.

Areas of Interest *(Please specify all that apply)*
- ☐ Cell Lysis
- ☐ Desalting
- ☐ Crosslinkers
- ☐ Protease Assay
- ☐ Protein Detection
- ☐ Protein Interaction

How do you currently prepare samples?
- ☐ Manual
- ☐ Automated (please specify the name of the company/model) _____

How do you isolate/purify your target protein?
- ☐ Agarose / Sepharose
- ☐ Affinity Supports
- ☐ Sample Prep
- ☐ Multiplex Protein Profiling
- ☐ IMAC
- ☐ Magnetic Affinity Purification

Do you have a KingFisher instrument in your lab?
- ☐ No
- ☐ Yes

If yes, please specify the model:
- ☐ KingFisher
- ☐ KingFisher mL
- ☐ KingFisher 96
- ☐ KingFisher Flex

How many samples do you analyze (quantities) per week?
- ☐ 1-30 samples/week
- ☐ 31-60 samples/week
- ☐ 61-90 samples/week
- ☐ 91-120 samples/week

Which downstream applications would you perform with isolated/purified protein(s) and the magnetic beads?
- ☐ Immunoprecipitation
- ☐ Therapeutic protein purification
- ☐ Phosphopeptide enrichment
- ☐ Western Blot Analysis
- ☐ SDS-PAGE
- ☐ MS analysis
- ☐ Other (Please specify) _____

Name _____ Title _____

Institution _____ Bldg./Rm. # _____

Address _____

City _____ State _____ Zip Code _____

Country _____

Phone ( ) _____ Fax ( ) _____

E-mail * _____

*By providing my email address, I acknowledge that I may occasionally receive new product announcements from the Thermo Fisher Scientific Inc. Pierce Protein Research Product Line via email.

For immediate information: Internet: www.thermo.com/pierce • Tel: 800-874-3723 or 815-968-0747 • Fax: 815-968-7316

1601697 08/08 AUTO

Speed and sample purity are major challenges for proteomics researchers. Additionally, the amount of work required for proteomic analysis necessitates higher sample- and data-processing throughput. The Thermo Scientific KingFisher® Flex Instrument and Pierce® Protein Research Products address these challenges, resolving all of your protein purification problems.

The KingFisher Flex Instrument can purify proteins ranging from simple recombinant proteins to complex therapeutic antibodies (Table 1). Based on state-of-the-art patented magnetic separation technology, KingFisher Systems enable you to process protein samples from virtually any source, including blood, cell cultures, tissue lysates and soil. Efficient operational speeds allow you to process up to 96 samples in as few as 15 minutes. The patented magnetic separation technology solves the problem of purifying recombinant proteins with highly complex structures and varying physio chemical properties. The Thermo Scientific BindIt™ Software enables you to develop your own robust protocols for other proteomics application workflows.

Table 1. Applications for Thermo Scientific Pierce Magnetic Beads.

Magnetic Beads	Applications and Sample Source	Recommended Detection Methods
Protein A	• Purify antibodies from serum, cell culture supernatant or ascite • Immunoprecipitate antigens from cell or tissue extracts	• SDS-PAGE • Western blot
Protein G	• Purify antibodies from serum, cell culture supernatant or ascites • Immunoprecipitate antigens from cell or tissue extracts	• SDS-PAGE • Western blot • Mass spectrometry
Glutathione	• Purify GST-fusion proteins from crude cell lysate prepared from bacteria, yeast, plant or mammalian cells	• SDS-PAGE
Titanium dioxide (Kit)	• Isolate and enrich phosphopeptides from complex biological samples	• Mass spectrometry

The new Thermo Scientific Pierce Magnetic Beads are available with high-quality Protein A, Protein G, glutathione and titanium dioxide for reliable and quick sample analysis with the KingFisher Flex Instrument.

Together, the KingFisher Flex Instrument and the Pierce Magnetic Beads enable routine purification of antibodies, antigens, pharmaceuticals and vaccines using any kind of host cell such as mammalian, insect, yeast, bacteria or transgenic animals, and plants. The automated fractionation is a valuable method for mass spectrometry analysis and biomarker discovery in which large numbers of patient samples are analyzed for reliable detection of disease-specific peptides or proteins. Fast and convenient protocols are available for automated and manual processing.

Contact Information

Belgium and Europe, the Middle East and Africa Distributors
Tel: +32 53 85 71 84

France
Tel: 0 800 50 82 15

The Netherlands
Tel: 076 50 31 880

Germany
Tel: 0228 9125650

United Kingdom
Tel: 0800 252 185

Switzerland
Tel: 0800 56 31 40

Email: perbio.euromarketing@thermofisher.com
www.thermo.com/perbio

United States
Tel: 815-968-0747 or 800-874-3723
Customer Assistance email: Pierce.CS@thermofisher.com
www.thermo.com

For other regions, visit www.thermo.com/pierce

Thermo Scientific Pierce Protein Reagents

Ordering Information

Product #	Description	Pkg. Size
88800	Protein A Magnetic Beads	1 ml
88801	Protein A Magnetic Beads	5 ml
88806	Protein G Magnetic Beads	1 ml
88807	Protein G Magnetic Beads	5 ml
88821	Glutathione Magnetic Beads	4 ml
88822	Glutathione Magnetic Beads	20 ml
88811	Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit Includes: TiO_2, Magnetic Beads, 20X, 1 ml Binding Buffer, 100 ml Washing Buffer, 25 ml Elution Buffer, 3 ml 96-well Skirted PCR Plate, 2 ea.	Kit
88812	Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit Includes: Magnetic TiO_2 Beads, 0.25 ml Binding Buffer, 100 ml Washing Buffer, 25 ml Elution Buffer, 3 ml 96-well Skirted PCR Plate, 2 ea.	Kit

For information on Pierce Protein Research Products, visit www.thermo.com/pierce

Thermo Scientific KingFisher Flex Products

Ordering Information

Product #	Description	Pkg. Size
5400610	KingFisher Flex 96 PCR head	1 ea.
5400620	KingFisher Flex 96 KF head	1 ea.
5400630	KingFisher Flex 96 deep well head	1 ea.
5400640	KingFisher Flex 24 deep well head	1 ea.
Consumables for KingFisher Flex Instrument		
97002514	KingFisher Flex 96 tip comb for PCR magnets for PCR magnets	80 pcs.
97002524	KingFisher Flex 96 tip comb for KF 96 magnets	100 pcs.
97002534	KingFisher Flex 96 tip comb for DW magnets	100 pcs.
97002610	KingFisher Flex 24 deep well tip comb and plate	50 pcs of each
97002540	KingFisher 96 KF plate (200µl)	48 pcs.
95040450	Microtiter Deepwell 96 Plate, V-bottom	50 pcs.
95040460	Microtiter Deepwell 96 Plate, V-bottom Sterile	50 pcs.
95040470	KingFisher Flex 24 Deep Well Plate	50 pcs.
95040480	KingFisher Flex 24 Deep Well Plate Sterile	50 pcs.

For more information or to order these or other KingFisher products, visit www.thermo.com/kingfisher

Thermo SCIENTIFIC

Pierce Protein Research Products
PO Box 117
Rockford, IL
61105-0117

Attention mailroom/recipient: Help keep our mail list current. Please fax (800-842-5007 or 815-968-8148) back this panel with your corrections or deletions made to the address.

1601697 08/08 Printed in the US

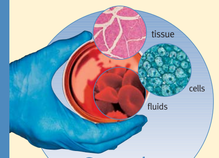
Thermo Scientific Automated Solutions for Proteomic Workflows

Part of Thermo Fisher Scientific

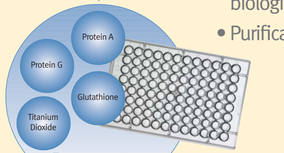
Thermo SCIENTIFIC

Accelerate proteomics throughput workflows with Thermo Scientific Automated Solutions

- Fully automated high-speed protein purification
- Wide-range of magnetic affinity chromatography beads
- Highest throughput sample processing capability available
- Efficient protein purification from complex biological samples (Figures 1-4)
- Purification protocols tailored to your application needs



Sample

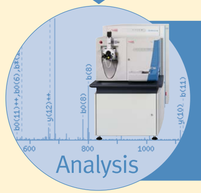


Reagents

Pierce Magnetic Beads
- Protein A
- Protein G
- Glutathione
- Titanium Dioxide

Purification

KingFisher Automated Purification
- Bind sample with beads
- Wash to get rid of impurities
- Elute purified product



Analysis

Sample Analysis Results

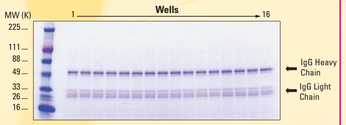


Figure 1. Highly reproducible IgG purification. Thermo Scientific Pierce Protein A Magnetic Beads (Product # 88800) and a KingFisher 96 Instrument was used to purify IgG from rabbit serum in 16 wells of a 96 deep-well plate. The beads (0.5 mg per well) were incubated 1 hour with 5 mg of serum, washed three times and eluted in 0.1 M glycine, pH 2.8. The eluates were boiled in SDS-PAGE reducing sample buffer, resolved by SDS-PAGE and stained with Thermo Scientific Imperial™ Protein Stain (Product # 24615). The KingFisher 96 instrument had excellent reproducibility across all 16 wells.

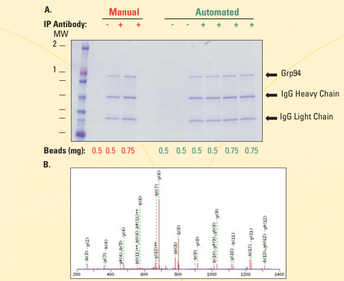


Figure 2. Manual and automated immunoprecipitation using Thermo Scientific Pierce Protein G Magnetic Beads produced similar results. Panel A. MOPC cell lysate (0.75 mg per sample) was combined with and without anti-Grp94 antibody (10 µg) and incubated overnight at 4°C. Pierce Protein G Magnetic Beads (Product # 88801) were added to a 96 deep-well plate (0.5 mg or 0.75 mg per well). Using a KingFisher 96 Instrument, the beads were washed with Tris-buffered saline containing 0.1% Tween™-20 Detergent, incubated 1 hour with the antigen sample/antibody mixture, washed three times and then eluted for 10 minutes at 96°C with SDS-PAGE reducing sample buffer. The same procedure was performed manually using a magnetic stand and microcentrifuge tubes. Eluates were resolved by SDS-PAGE and stained with Imperial Protein Stain (Product # 24615). Results with both manual and automated protocols were similar. The Pierce Protein G Magnetic Beads had low nonspecific binding. **Panel B.** The Grp94 gel band was excised, digested with trypsin and analyzed on the LTQ XL Mass Spectrometer. The Grp94 protein was identified with 39% sequence using the Thermo Scientific Mascot Database. An MS/MS spectrum of a representative peptide (GVVDSDDLPLNVSR) is shown.

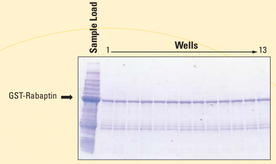


Figure 3. Purification of GST-Rabaptin using Thermo Scientific Pierce Glutathione Magnetic Beads. Pierce Glutathione Magnetic Beads (100 µl) were aliquoted into a 96 deep well plate in combination with Binding/Wash Buffer (125 mM Tris, 150 mM NaCl, pH 8) to a final volume of 200 µl. Protein purification was performed using the KingFisher 96 Instrument. Briefly, beads were washed in Binding/Wash Buffer, incubated 1 hour with bacterial cell lysate, washed three times in Binding/Wash Buffer and eluted with 50 mM reduced glutathione prepared fresh in Binding/Wash Buffer. Eluates were boiled in SDS-PAGE reducing sample buffer, resolved on an SDS-PAGE gel and stained with Imperial Stain. The Glutathione Magnetic Beads were found to isolate GST-Rabaptin reproducibly across 13 wells.

KingFisher Technology enables you to purify proteins with the speed and accuracy no other technology can match. By shifting magnetic particles through purification phases, you achieve excellent results without cross-contamination or reagent carryover. KingFisher Instruments offer scientists highly versatile, automated magnetic particle processing for protein from virtually any source.

The Thermo Scientific KingFisher Flex Instrument is the newest member of our magnetic particle processor family. High flexibility and throughput with the incorporation of magnetic heads for both 96- and 24-well plates provides excellent reproducibility and quality.

For information on Thermo Scientific KingFisher Instruments, visit www.thermo.com/kingfisher

The Thermo Scientific LTQ XL™ Mass Spectrometer is the only instrument that offers multiple dissociation techniques for proteomics research applications. The LTQ XL extends the legendary MS^n performance of the original Thermo Scientific LTQ Instrument, incorporating more techniques to generate structural information through innovations in ion trap technology.

The Thermo Scientific LTQ Orbitrap™ Mass Spectrometer enables fast, reliable detection and identification of protein in complex mixtures. Routine high-resolution and mass measurement accuracy of 2-3 ppm provides excellent data for proteomic research applications. The LTQ Orbitrap Mass Spectrometer is the most comprehensive solution for complex post-translational modifications analysis, intelligent sequencing of peptides, top-down and middle-down analysis, and protein quantitation via stable isotope labeling, such as Thermo Scientific TMT Isobaric Tandem Mass Tags, or label-free quantitation.

For more information on the Thermo Scientific LTQ Orbitrap Mass Spectrometer, visit www.thermo.com/orbitrap

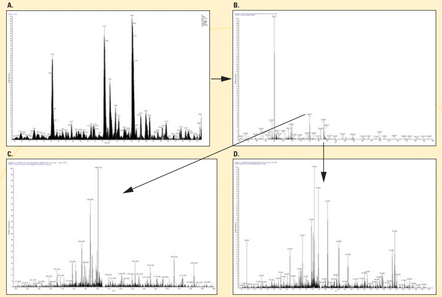


Figure 4. High resolution LC-MS/MS data obtained from 2 mg of a tryptic peptide digest prepared from peripheral blood mononuclear cells (lymphocytes) enriched with the Thermo Scientific Pierce TiO$_2$ Phosphopeptide Enrichment Kit. Peptide samples were processed on a KingFisher 96 Instrument. **A.** Full scan chromatogram obtained on an LTQ-FT Ultra high-resolution mass spectrometer, resolution 200K, mass accuracy < 2ppm. **B.** Zoom of one full scan, retention time 64.50 minutes. **C.** MS2 fragmentation spectrum of single phosphopeptide ion, parent mass 1582.70. Peptide sequence: SS*PFKVS*PLTFGR. The protein was identified as serum deprivation response protein. **D.** MS2 fragmentation spectrum of a single phosphopeptide ion, parent mass 1722.80. The peptide sequence is LPS*GSGAASPTGSAVDIR. The protein was identified as AHNAK nucleoprotein isoform 1. * = Site of phosphorylation

Table 2. Summary of the Figure 4 data obtained from sample enriched and not enriched with the Thermo Scientific Pierce TiO$_2$ Phosphopeptide Enrichment Kit.

	Enriched	Non-Enriched
Total number of proteins identified	185	247
Total number of phosphoproteins identified	160	1
Total number of peptides identified	2347	2457
Total number of phosphopeptides identified	2009	7
Total number of unique phosphopeptides identified	177	1
Relative enrichment for phosphopeptides (%)	86	0.3

Thermo SCIENTIFIC

BUSINESS REPLY MAIL
FIRST CLASS MAIL PERMIT NO. 1294 ROCKFORD, IL
POSTAGE WILL BE PAID BY ADDRESSEE

Thermo SCIENTIFIC
PIERCE PROTEIN RESEARCH PRODUCTS
PO BOX 117
ROCKFORD IL 61105-9976

NO POSTAGE NECESSARY IF MAILED IN THE UNITED STATES