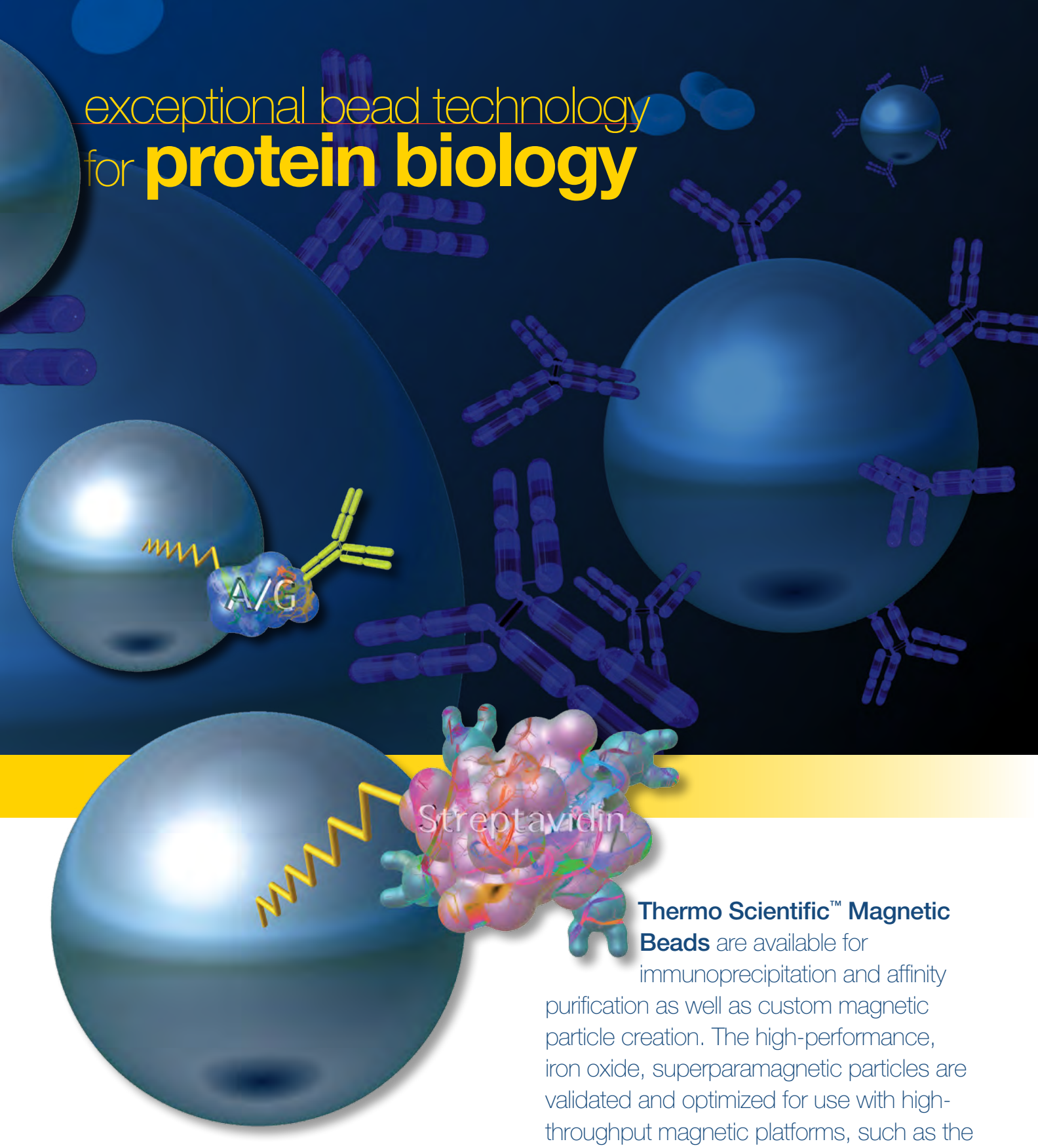
Thermo Scientific
Magnetic Bead Technology

magnetic bead technology
for better assay development

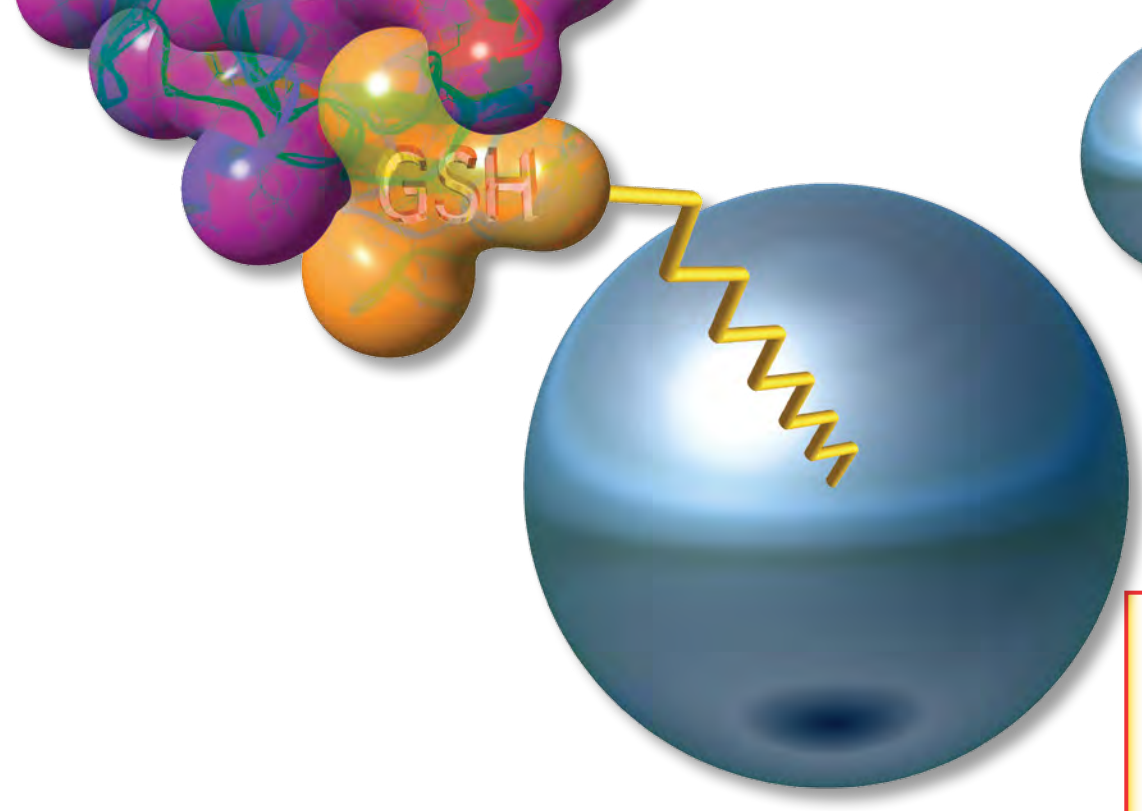
custom magnetic particles • immunoprecipitation • affinity purification • ChIP

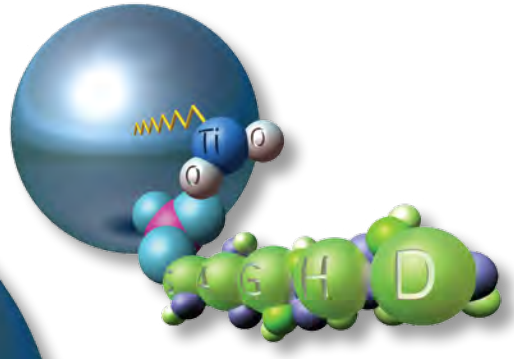
Thermo SCIENTIFIC

exceptional bead technology for **protein biology**

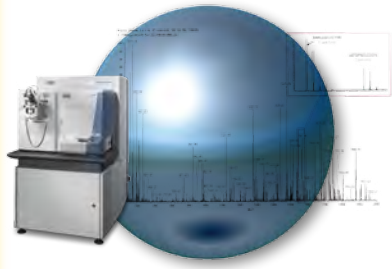


Thermo Scientific™ Magnetic Beads are available for immunoprecipitation and affinity purification as well as custom magnetic particle creation. The high-performance, iron oxide, superparamagnetic particles are validated and optimized for use with high-throughput magnetic platforms, such as the Thermo Scientific™ KingFisher™ Duo and Flex Instruments. Samples can be analyzed by Western blotting or on Thermo Scientific™ Mass Spectrometers for quantitation of low abundant targets.

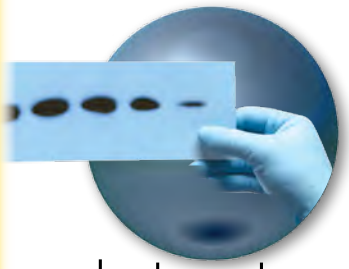




identify and quantitate



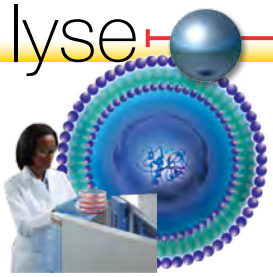
Identify and quantitate your analyte using Thermo Scientific Mass Spectrometers and labeling reagents



detect

Use our highly sensitive substrates for Western blot detection

lyse



Prepare cells, tissue or fluid samples with Thermo Scientific™ Lysis Reagents

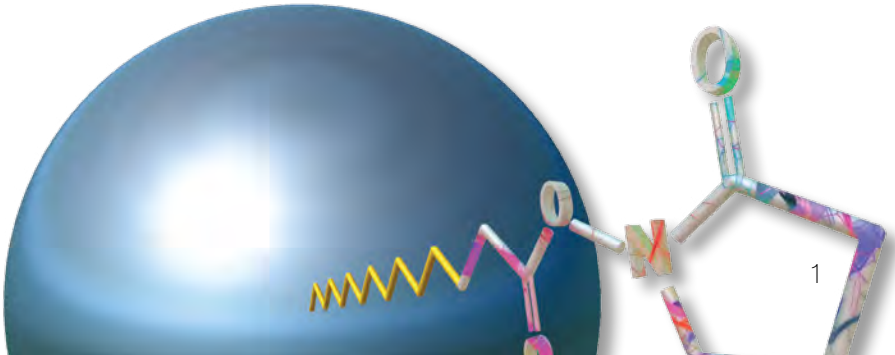
target

Isolate your target with one of our magnetic beads:

- **Activated magnetic beads** for creating custom affinity resins .. pg. 2
- **Magnetic Protein A/G/L** for immunoprecipitation ..... pg. 4
- **Validated kits** for immunoprecipitation, ChIP or RNA-pull downs ..... pg. 16
- **Resins** for protein enrichment ..... pg. 20

isolate

Choose to run a few samples on the bench or create a high-throughput assay with KingFisher Instruments



immobilize your ligand
for custom magnetic assays

Magnetic Protein Immobilization Beads

Thermo Scientific™ Pierce™ NHS-Activated Magnetic Beads enable covalent, amine-based conjugation of proteins to magnetic beads in a simple mix-and-go format for use in custom affinity purification experiments. The activated magnetic beads contain *N*-hydroxysuccinimide (NHS) functional groups that react with primary amines, forming stable amide linkages. Once they are covalently attached, the immobilized proteins are highly resistant to leaching from the bead surface. When prepared beads are used in experiments, nonspecific binding is negligible because nonreacted NHS-ester groups are thoroughly blocked during the coupling procedure.

Highlights

- **High capacity** – at least four times greater binding capacity than NHS-activated magnetic beads from other suppliers
- **Easy to use** – immobilize in a simple one-step reaction with minimal hands-on time
- **Safe** – no hazardous chemicals (e.g., sodium cyanoborohydride and cyanogen bromide) needed
- **Ligand compatible** – use with nearly any primary amine-containing compound or affinity ligand to immobilize
- **Low nonspecific binding** – the bead surface is pre-blocked and any nonreacted NHS-ester groups are fully quenched
- **Protocol compatible** – protein coupling to the beads and downstream applications can be performed manually or by automation (e.g., KingFisher Instruments)

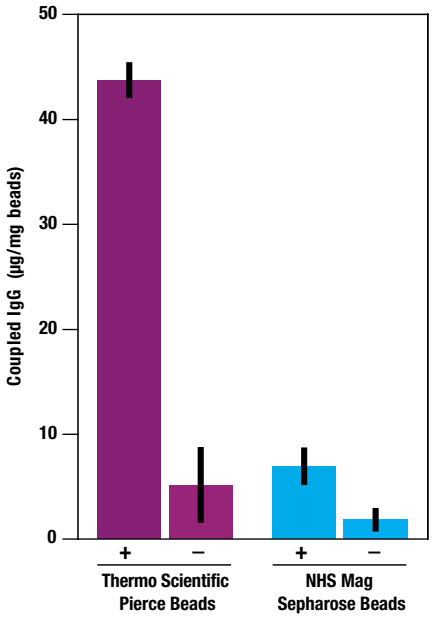


Figure 1. Significantly better coupling capacity with Thermo Scientific Pierce NHS-Activated Magnetic Beads. Rabbit IgG (1mg/mL) was coupled in PBS for two hours at pH7.2 to 3mg each of Pierce NHS-Activated Magnetic Beads and NHS Mag Sepharose™ Beads (GE Life Sciences). Negative control beads (–) were prepared by quenching or blocking using respective manufacturer protocols. Bound protein was measured using the Thermo Scientific™ Pierce™ 660nm Protein Assay by subtracting the amount of protein in the flow-through from the amount loaded. The Pierce NHS-Activated Magnetic Beads coupled more than four times as much protein as the equivalent amount of NHS Mag Sepharose Beads.

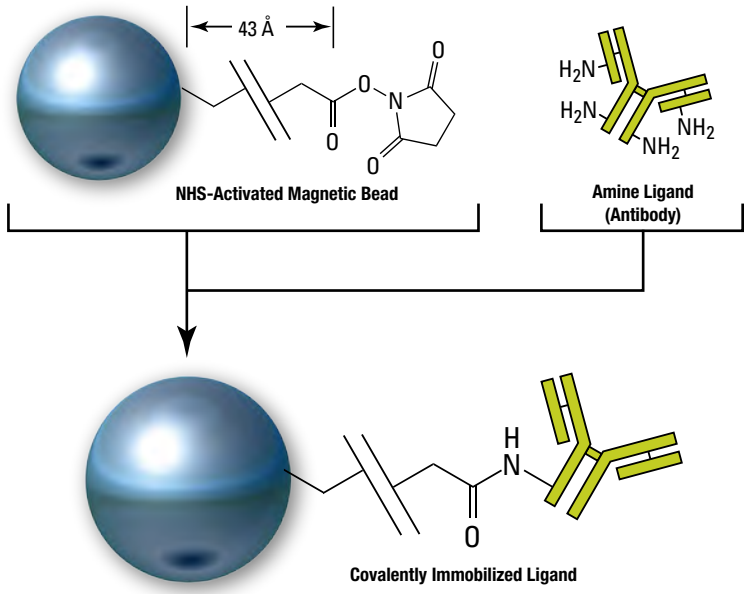
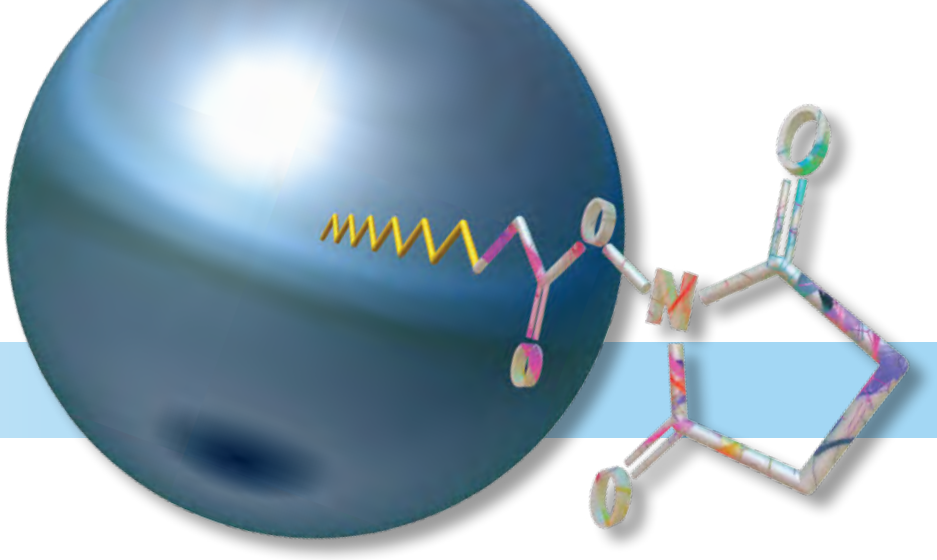


Figure 2. Reaction scheme for conjugation of protein onto Thermo Scientific Pierce NHS-Activated Magnetic Beads.



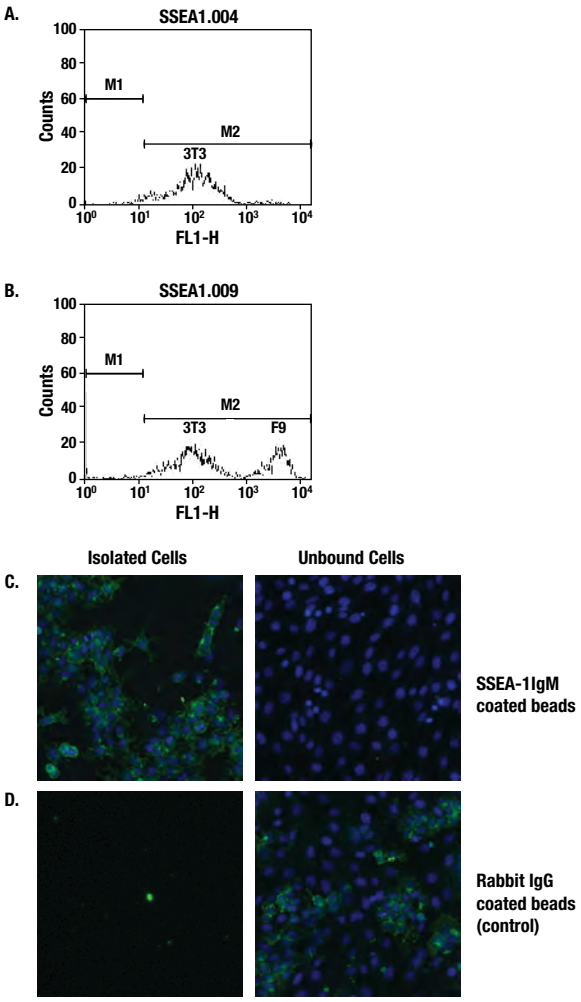


Figure 3. Effective cell separation. Pierce NHS-Activated Beads were coated with Thermo Scientific™ Stage-specific Embryonic Antigen 1 (SSEA-1) mouse IgM or rabbit IgG as a negative control. The beads were incubated with a 50:50 co-culture of F9 mouse embryonal carcinoma cells (SSEA-1 positive) and NIH 3T3 cells (SSEA-1 negative) for 20 minutes at 4°C. The beads were collected on a magnetic stand and the unbound cell fraction was evaluated by flow cytometry using anti-SSEA-1 mouse IgM and goat anti-mouse IgM-fluorescein. **Panel A** shows F9 cells were selectively depleted with anti-SSEA-1-coated NHS- activated magnetic beads. **Panel B** shows neither cell type bound to the negative control (rabbit IgG-coated NHS magnetic beads). Both the bead-bound and unbound cell fractions were cultured for 24 hours, fixed and then stained with mouse anti-SSEA-1 antibody, Thermo Scientific™ DyLight™ 488 conjugated goat anti-mouse IgM and Hoechst™ nuclear stain. Cells were visualized using the Thermo Scientific™ ToxInsight™ Platform. **Panel C** shows that the SSEA-1 antibody coated magnetic NHS beads effectively separated the F9 cells from the NIH 3T3 cells. As expected, the rabbit-IgG-coated magnetic NHS beads **(Panel D)** did not bind either cell type, and the corresponding unbound fraction contained a 50:50 ratio of both cell types.

Table 1. Properties of Thermo Scientific Pierce NHS-Activated Magnetic Beads.

Composition	*N*-hydroxysuccinimide (NHS) functional groups on a blocked magnetic bead surface
Magnetization	Superparamagnetic (no magnetic memory)
Mean Diameter	1µm (nominal)
Density	2.0g/cm^3
Bead Concentration	10mg/mL in DMAC
Binding Capacity	≥26µg of rabbit IgG/mg of beads

Ordering Information

Product #	Description	Pkg. Size
88826	**Pierce NHS-Activated Magnetic Beads** *Sufficient for: Binding ≥ 26µg of rabbit IgG/mg of beads*	1mL
88827	**Pierce NHS-Activated Magnetic Beads** *Sufficient for: Binding ≥ 26µg of rabbit IgG/mg of beads*	5mL

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Magnetic Immunoprecipitation Beads

The Thermo Scientific™ Pierce™ Protein A/G Magnetic Bead is a single particle that is compatible with all commonly used antibodies for immunoprecipitation (IP). These beads are coated with genetically engineered Protein A/G, a recombinant protein that combines the IgG binding domains of both Protein A and Protein G. This combination enables the capture of antibodies from a wider range of species and isotypes than either protein alone. Using our crosslinker chemistry, you can immobilize an antibody onto the magnetic particle and prevent IgG contamination in your immunoprecipitated sample.

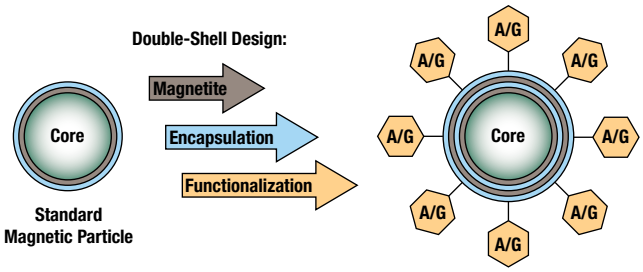
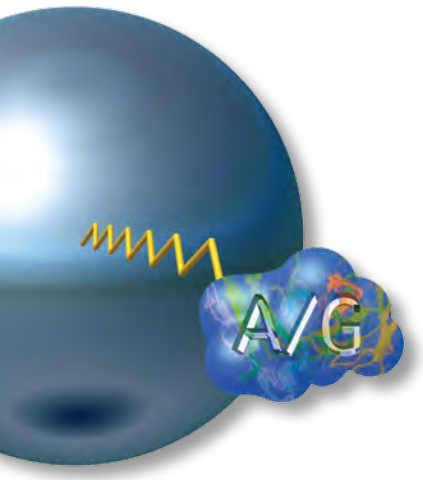


Figure 4. Diagram of Thermo Scientific Pierce Protein A/G Magnetic Beads. The magnetic particles are 1µm in diameter and are specially manufactured with two layers of magnetite and encapsulation. Recombinant Protein A/G is coupled to the bead surface.



Highlights

- **Compatible** – one magnetic bead type that can capture most primary antibodies
- **Fast** – immunoprecipitating in as few as 30 minutes helps reduce nonspecific binding and improves the capture of transient protein complexes
- **Clean** – immobilize your antibody to prevent contamination in your eluate
- **Resistant** – no leaching of Protein A/G in the presence of detergents, low pH buffers or common mass spectrometry solvents
- **Efficient** – immunoprecipitate with half the recommended volume of magnetic particles compared to other magnetic beads

Table 2. Properties of Thermo Scientific Pierce Magnetic Protein A/G Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a monolayer of recombinant Protein A/G
Mean Diameter	1µm (nominal)
Density	2.0g/cm^3
Bead Concentration	10mg/mL in water with sodium azide
Binding Capacity	55 to 85µg of rabbit IgG/mg magnetic beads

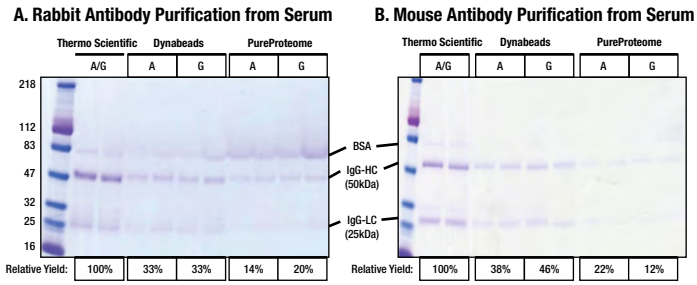
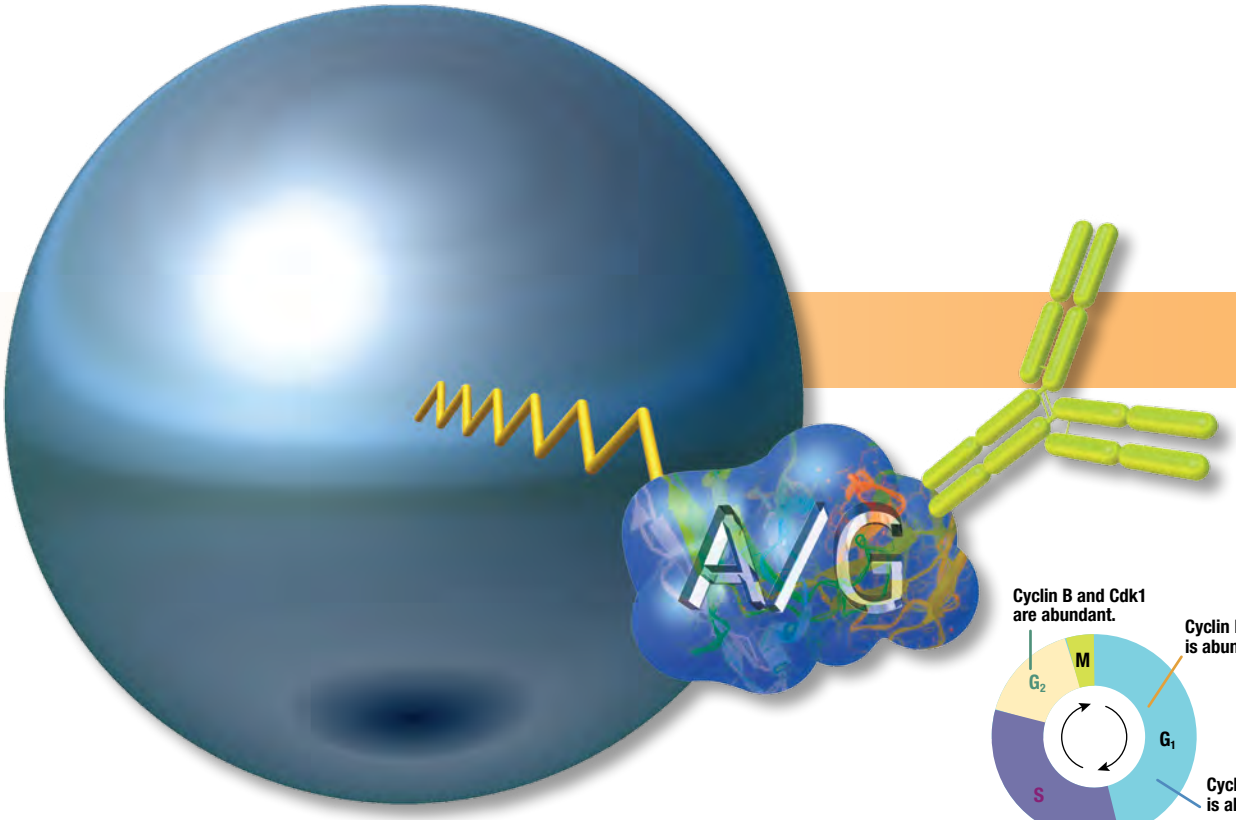


Figure 5. Thermo Scientific Pierce Protein A/G Magnetic Beads isolate significantly more IgG from rabbit and mouse serum with less background than other brands of Protein A and Protein G magnetic particles. Using a KingFisher Flex Instrument with a 96 deep well plate, IgG was purified from 5mg of rabbit and mouse serum using 50µL of Pierce Protein A/G Magnetic Beads, Dynabeads™ Protein A or G (Life Technologies), or PureProteome™ Protein A or G Beads (Millipore). The beads were washed with Tris-buffered saline containing 0.05% Tween™-20 (TBST), incubated for one hour with serum diluted in TBST, washed three times, and then eluted with 0.1M glycine, pH 2.8 for 10 minutes at room temperature. The eluates were resolved by SDS-PAGE and stained with Thermo Scientific™ Imperial™ Protein Stain. **Panel A:** Rabbit serum; **Panel B:** Mouse serum. The IgG heavy chain bands were quantified by densitometry. The values for each set of duplicate bands were averaged and expressed as a percentage of the average for the Pierce Protein A/G Magnetic Beads.



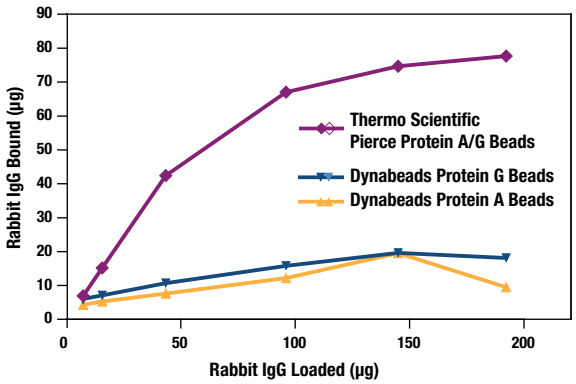


Figure 6. The rabbit IgG binding capacity of Thermo Scientific Pierce Protein A/G Magnetic Beads is approximately four times greater than that of other Protein A and Protein G beads. Pierce Protein A/G Magnetic Beads or Dynabeads Protein A or Protein G (Life Technologies) were added to a 96 deep-well plate (1mg beads per well). Using the Thermo Scientific™ KingFisher™ 96 Instrument, the beads were incubated for one hour with varying amounts of purified rabbit IgG (20 to 200µg). After binding, the samples were eluted at 96°C with SDS-PAGE reducing sample buffer. Binding was calculated using the Thermo Scientific™ Pierce™ BCA Protein Assay.

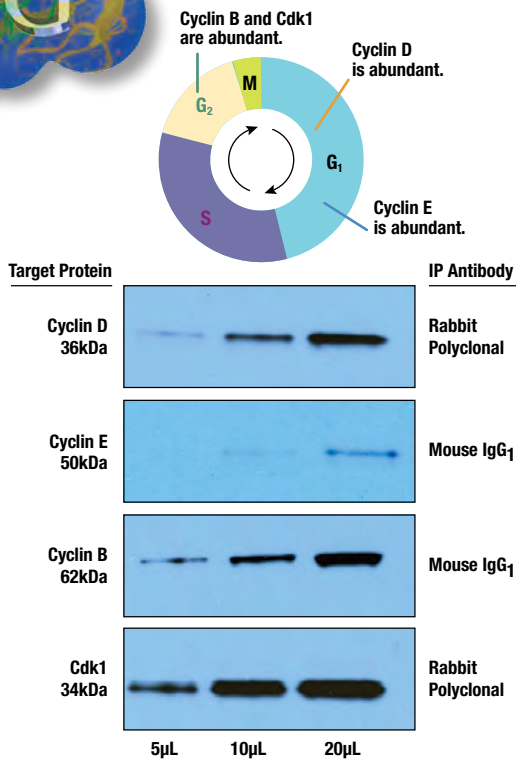


Figure 7. The Thermo Scientific Pierce Protein A/G Magnetic Beads effectively immunoprecipitate cell cycle proteins Cyclin D, Cyclin E, Cyclin B and Cdk1. U2OS (human osteosarcoma) cells were synchronized at G_0 followed by growth in 20% fetal bovine serum for 4, 6 and 18 hours before harvest. The cells were lysed in IP lysis/wash buffer, and 0.75mg of lysate (per sample) was incubated with anti-Cyclin D (rabbit polyclonal), anti-Cyclin E (mouse IgG_1), anti-Cyclin B (mouse IgG_1) or anti-Cdk1 (rabbit polyclonal) antibodies overnight at 4°C. The Pierce Protein A/G Magnetic Beads were added (50µL each) to a 96 deep-well plate and immunoprecipitations were performed using the KingFisher Flex Instrument. Eluted sample volumes of 5µL, 10µL and 20µL were resolved by SDS-PAGE and analyzed by Western blot.

Ordering Information

Product #	Description	Pkg. Size
88802	Pierce Protein A/G Magnetic Beads *Sufficient for: Binding 55 to 85µg rabbit IgG/mg beads.*	1mL
88803	Pierce Protein A/G Magnetic Beads *Sufficient for: Binding 55 to 85µg rabbit IgG/mg beads.*	5mL

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Magnetic Immunoprecipitation Beads

Thermo Scientific™ Pierce™ Protein A Magnetic Beads are used for immunoprecipitating antigens from cell or tissue extracts as well as purifying antibody from serum, cell culture supernatant or ascites fluid. Protein A can bind to antibodies from many different species, including mouse, human, rabbit, pig, dog and cat. The protocol for Pierce Protein A Beads is optimized for high recovery and high purity of isolated antibodies or antigens.

Highlights

- **Low nonspecific binding** – stable, pre-blocked beads provide clean purification product
- **Consistency** – magnetic beads eliminate resin loss and provide for more efficient separation of solutions than traditional IP methods that use only microcentrifuge tubes
- **Compatibility** – beads are compatible with manual and automated applications (e.g., KingFisher Instruments)

Table 3. Properties of Thermo Scientific Pierce Magnetic Protein A Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a monolayer of recombinant Protein A
Mean Diameter	1µm (nominal)
Density	2.0g/cm^3
Bead Concentration	10mg/mL in water with sodium azide
Binding Capacity	≥40µg of rabbit IgG/mg of beads; ≥400µg of rabbit IgG/mL of beads

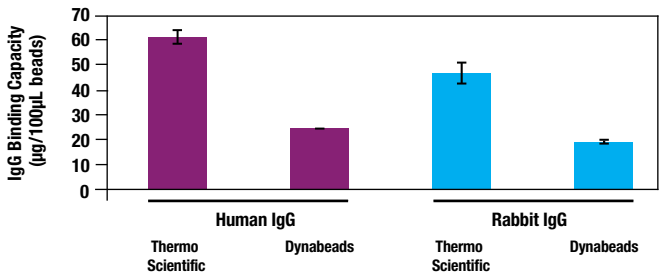


Figure 8. The human and rabbit IgG binding capacities of Thermo Scientific Pierce Protein A Magnetic Beads are approximately 2-fold higher than LifeTech Dynabead Protein A. Pierce Protein A Magnetic Beads or Dynabeads A (Life Technologies) were added to a 96 deep-well plate (100µL beads per well). Using the KingFisher Flex Instrument, the beads were incubated for one hour with 400µg purified human or rabbit IgG. Binding was calculated using the Pierce BCA Protein Assay by subtracting the amount of IgG in the flow-throughs from the IgG loaded.

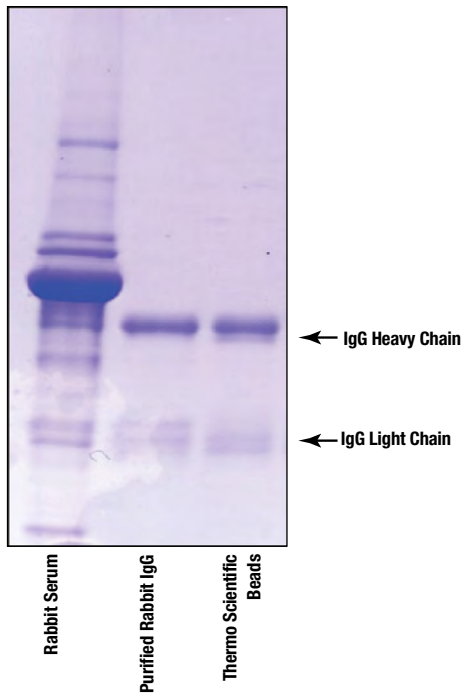
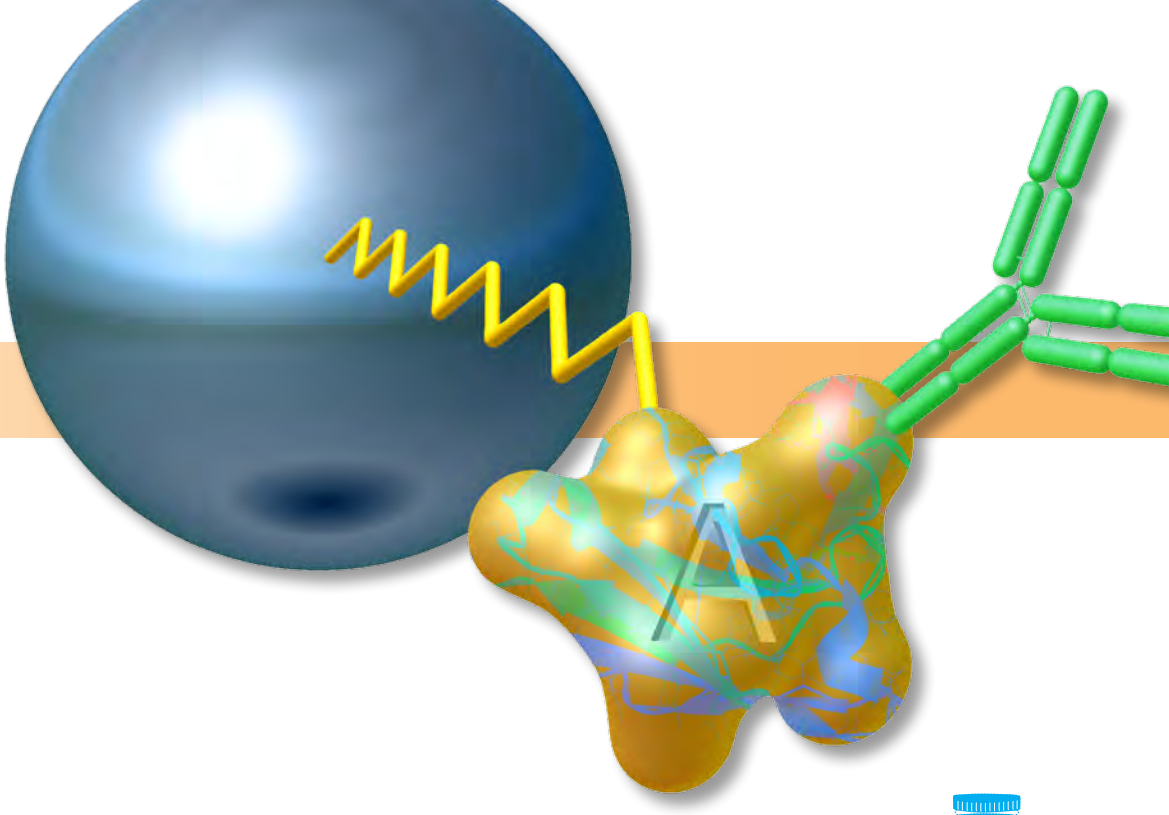
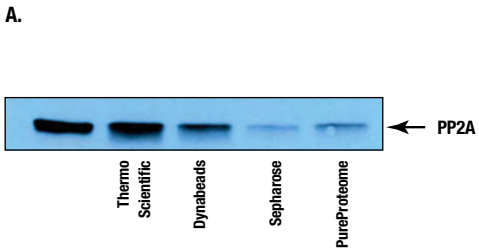


Figure 9. Thermo Scientific Pierce Protein A Magnetic Beads exhibit low nonspecific binding. Using a KingFisher Flex Instrument with a 96 deep-well plate, IgG was purified from 2mg of rabbit serum using 50µL of Pierce Protein A Magnetic Beads. The beads were incubated for one hour with serum diluted in phosphate-buffered saline containing 0.025% Tween-20 (PBST), washed twice with PBST and once with water, and then eluted with 0.1M glycine, pH 2.0 for 10 minutes at room temperature. The eluates were resolved and stained with Imperial Protein Stain. No serum proteins other than antibody heavy and light chains were detected in the eluted sample.





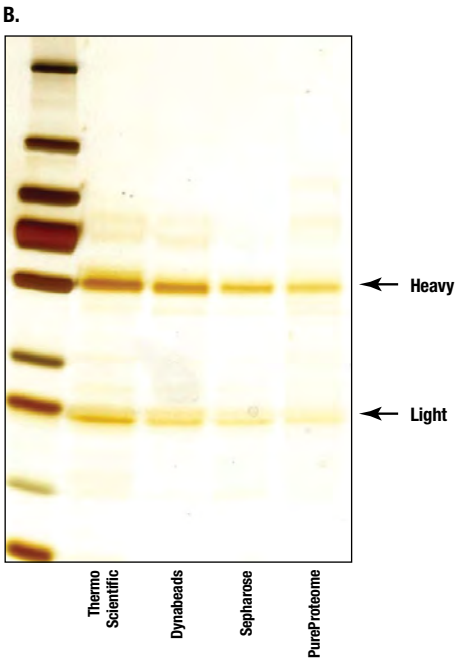


Figure 10. Better immunoprecipitation results with Thermo Scientific Pierce Protein A Magnetic Beads. PP2Aantibody (5µg) was incubated overnight at 4˚C with 0.5mg of A549 cell lysate. Using the KingFisher Flex Instrument, 50µL each of Pierce Protein A Magnetic Beads, Dynabeads A (Life Technologies), Protein A Magnetic Sepharose (GE Life Sciences) and PureProteome Protein A Beads (EMD/Millipore) were added to 96 deep-well plates. The beads were incubated for one hour with the antigen/antibody complex at room temperature, washed twice in phosphate-buffered saline containing 0.05% Tween-20, washed once in water and then eluted in 0.1M glycine, pH 2.0. Samples were resolved by SDS-PAGE and analyzed for Western blot for PP2A (**Panel A**) and by silver stain for nonspecific binding (**Panel B**). The Pierce Protein A Magnetic Beads were found to have higher yield of PP2A than other Protein A beads. Nonspecific binding was negligible for all beads tested.

Ordering Information

Product #	Description	Pkg. Size
88845	Pierce Protein A Magnetic Beads	1mL
88846	Pierce Protein A Magnetic Beads	5mL

build your own
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Magnetic Immunoprecipitation Beads

Thermo Scientific™ Pierce™ Protein G Magnetic Beads are high-capacity and high-throughput affinity particles for antibody purification and immunoprecipitation methods using manual or robotic magnetic separators. Protein G can bind to antibodies from many different species, including mouse, human, rabbit, cow, goat and sheep.

Highlights

- **High IP efficiency** – highest antibody yield
- **Low nonspecific binding** – stable, pre-blocked beads provide clean purification product
- **Assay consistency** – magnetic beads eliminate resin loss
- **High throughput** – compatible with manual and automated applications (e.g., KingFisher Instruments)

Table 4. Properties of Thermo Scientific Pierce Magnetic Protein G Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a monolayer of recombinant Protein G
Mean Diameter	1µm (nominal)
Density	2.0g/cm^3
Bead Concentration	10mg/mL in water with sodium azide
Binding Capacity	≥60µg of rabbit IgG/mg of beads; ≥600µg of rabbit IgG/mL of beads

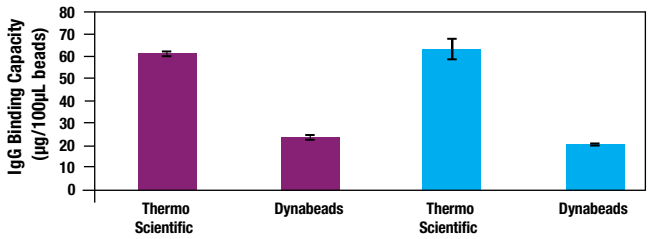


Figure 11. The human and rabbit IgG binding capacities of Thermo Scientific Pierce Protein G Magnetic Beads are approximately 3-fold higher than Dynabeads Magnetic Beads. Pierce Protein G Magnetic Beads and Dynabeads G (Life Technologies) were added to a 96 deep-well plate (100µL beads per well). Using the KingFisher Flex Instrument, the beads were incubated for one hour with 400µg purified human or rabbit IgG. Binding was calculated using the Pierce BCA Protein Assay by subtracting the amount of IgG in the flow-throughs from the IgG loaded.

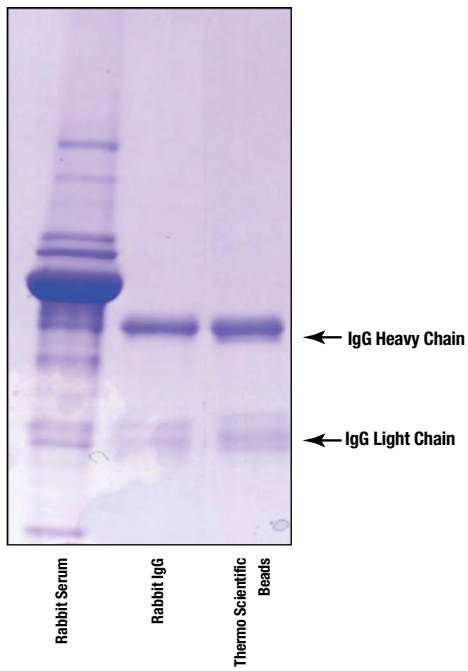
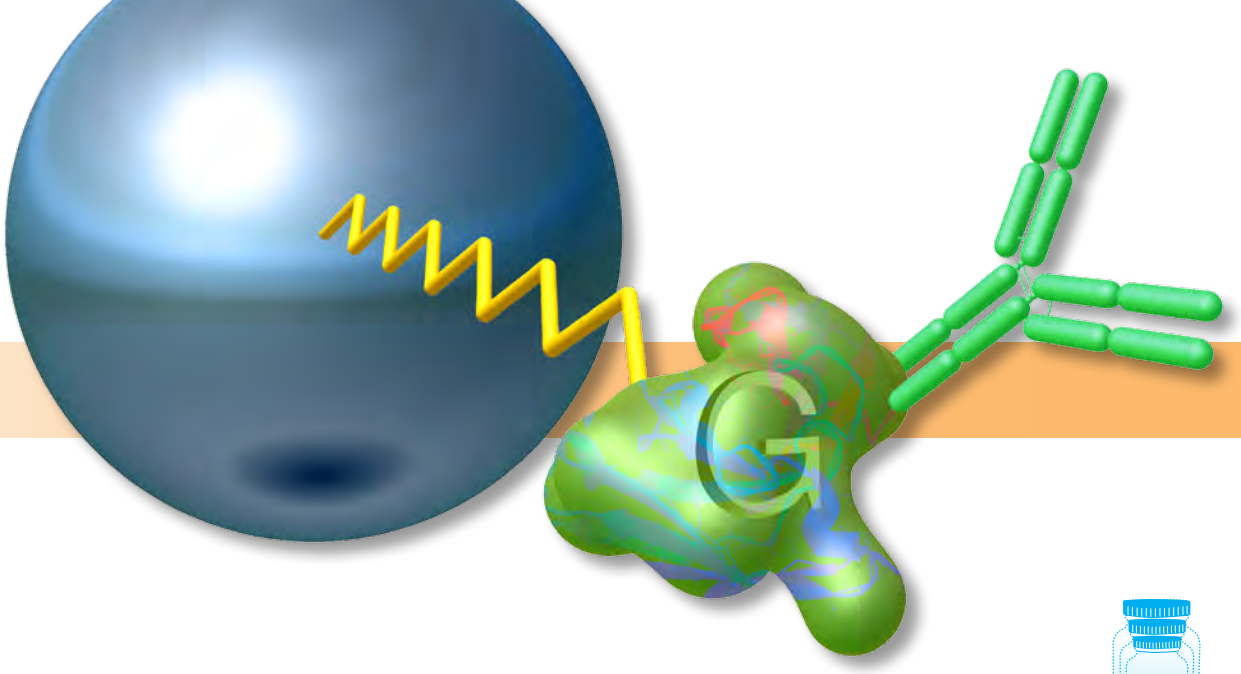
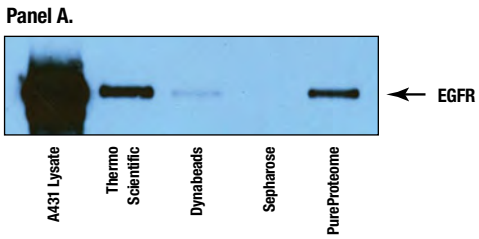


Figure 12. Thermo Scientific Pierce Protein G Magnetic Beads exhibit low nonspecific binding. Using a KingFisher Flex Instrument with a 96 deep-well plate, IgG was purified from 2mg of rabbit serum using 50µL of Pierce Protein G Magnetic Beads. The beads were incubated one hour with serum diluted in phosphate-buffered saline containing 0.025% Tween-20 (PBST), washed twice with PBST and once with water, and then eluted with 0.1M glycine, pH 2.0 for 10 minutes at room temperature. The eluates were resolved and stained with Imperial Protein Stain. No serum proteins other than antibody heavy and light chains were detected in the eluted sample.





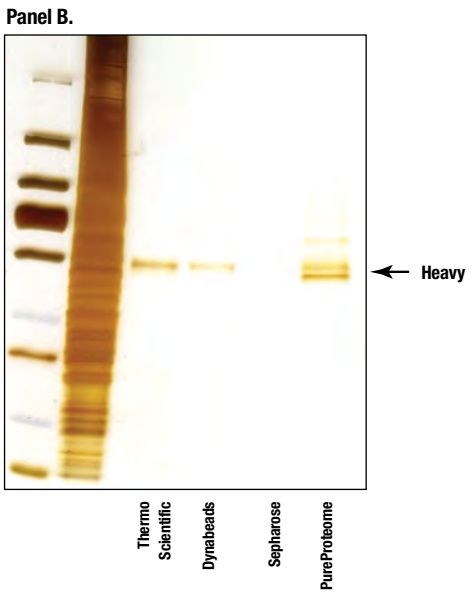


Figure 13. Better immunoprecipitation results with Thermo Scientific Pierce Protein G Magnetic Beads. EGF Receptor antibody (5µg) was incubated overnight at 4˚C with 0.75mg of A431cell lysate. Using the KingFisher Flex Instrument, 25µL each of Pierce Protein G Magnetic Beads, Dynabeads G (Life Technologies), Protein G Magnetic Sepharose (GE Life Sciences) and PureProteome Protein G Beads (EMD/Millipore) were added to 96 deep-well plates. The beads were incubated for one hour with the antigen/antibody complex at room temperature, washed twice in phosphate-buffered saline containing 0.05% Tween-20, washed once in water and then eluted in 0.1M glycine, pH 2.0. Samples were resolved by SDS-PAGE and analyzed by Western blot for EGFR (**Panel A**) and by silver stain for nonspecific binding (**Panel B**). The Pierce Protein G Magnetic Beads were found to have higher yield of EGFR than other Protein G beads. Nonspecific binding was negligible for all beads tested.

Ordering Information

Product #	Description	Pkg. Size
88847	Pierce Protein G Magnetic Beads	1mL
88848	Pierce Protein G Magnetic Beads	5mL

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Magnetic Immunoprecipitation Beads

Thermo Scientific™ Pierce™ Protein L Magnetic Beads are ideal for selective isolation of antibodies possessing kappa light chains. Protein L selectively binds mouse and human antibodies through kappa light chains and is commonly used to purify monoclonal antibodies in cell culture supernantants supplemented with bovine serum as Protein L does not bind bovine IgG. Protein L can bind a broader range of Ig classes than Protein A or Protein G, including IgG, IgM, IgA, IgE and IgD. Protein L binds strongly to human (kappa I, III and IV only), mouse (kappa I only), rat and pig immunoglobulins. It binds weakly to rabbit immunoglobulins and does not bind to immunoglobulins from bovine, goat or sheep. Single-chain variable fragments (scFv) and Fab fragments also bind to Protein L.

Highlights

- **Selective** – ideal for selective purification of human and mouse antibodies that have kappa light chains
- **Low nonspecific binding** – stable, pre-blocked beads provide clean purification of antibody
- **Compatibility** – beads are compatible with manual and automated applications (e.g., KingFisher Instruments)

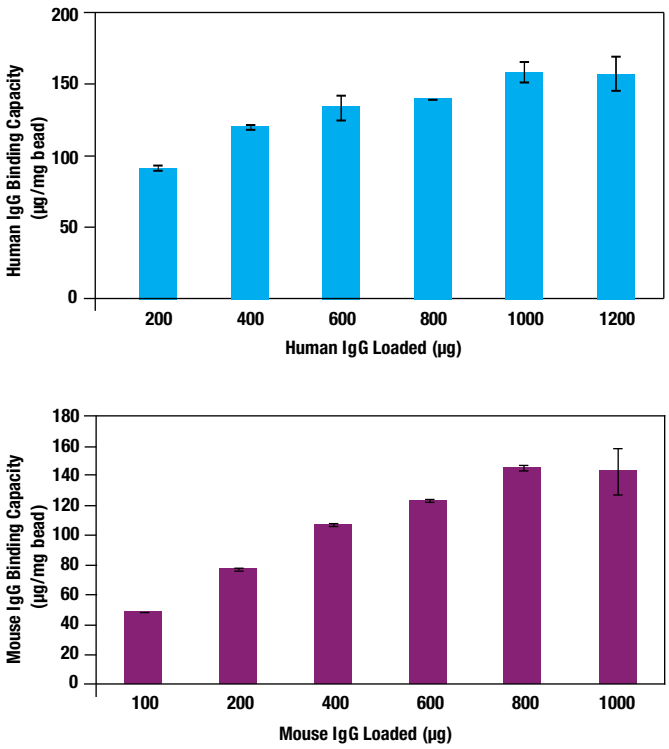
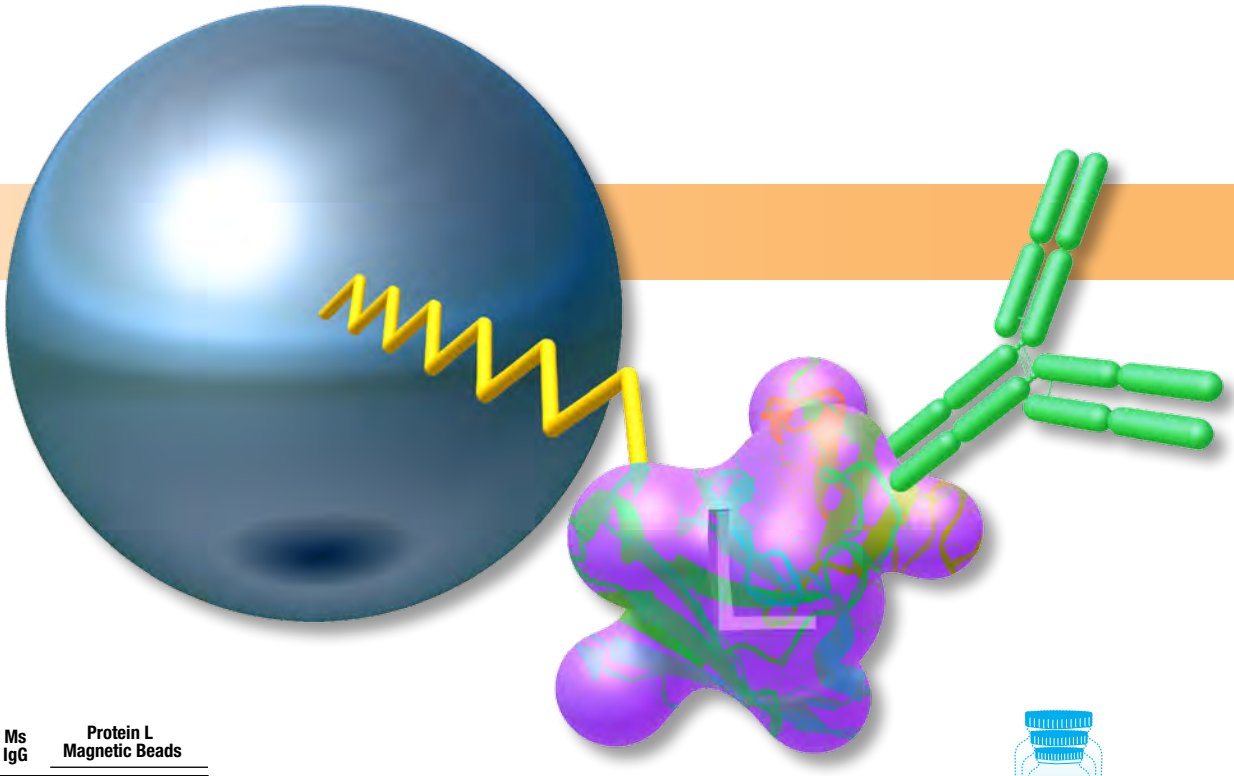
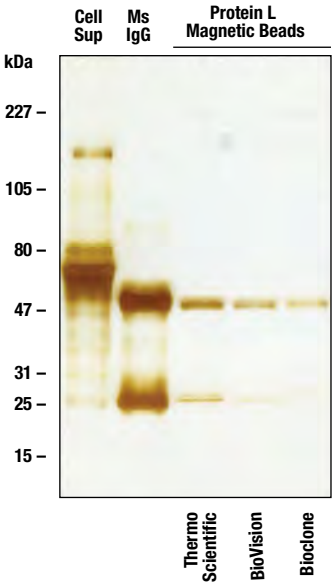


Figure 14. Human and mouse IgG binding capacity curves for Thermo Scientific Pierce Protein L Magnetic Beads. Pierce Protein L Magnetic Beads were added to a 96 deep-well plate (100µL beads per well). Using the KingFisher Flex Instrument, the beads were incubated for one hour with purified human or mouse IgG (amounts shown in graphs). Binding was calculated using the Pierce BCA Protein Assay by subtracting the amount of IgG in the flow-throughs from the IgG loaded.

Table 5. Properties of Thermo Scientific Pierce Magnetic Protein L Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a monolayer of recombinant Protein L
Mean Diameter	1µm (nominal)
Density	2.0g/cm^3
Bead Concentration	10mg/mL in water with sodium azide
Binding Capacity	≥110µg of human IgG/mg of beads; ≥1.1mg of human IgG/mL of beads





Ordering Information

Product #	Description	Pkg. Size
88849	Pierce Protein L Magnetic Beads	1mL
88850	Pierce Protein L Magnetic Beads	5mL

Figure 15. Thermo Scientific Pierce Protein L Magnetic Beads isolate more mouse IgG from cell culture supernatant than other suppliers' Protein L magnetic beads. Using a KingFisher Flex Instrument with 96 deep-well plates, IgG was purified from cell culture supernatant using 50µL each of Pierce Protein L Magnetic Beads, and Protein L Magnetic Beads from BioVision and Bioclone. The beads were incubated for one hour with undiluted cell culture supernatant containing 0.025% Tween-20, washed twice with PBST and once with water, and then eluted with 0.1M glycine, pH 2.0 for 10 minutes at room temperature. The eluates were resolved and stained with the Thermo Scientific™ Pierce™ Silver Stain Kit. Note that binding of mouse IgG with Protein L only occurs when kappa light chains are present. All of the beads were found to have negligible nonspecific binding.

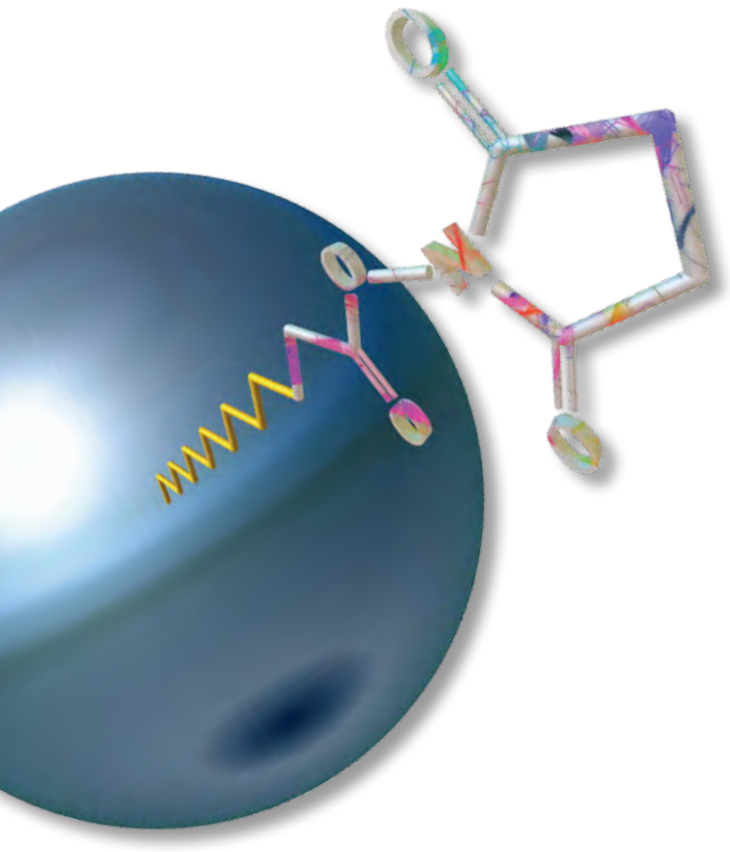
select an easy-to-use **validated kit**

Magnetic Immunoprecipitation (IP) Kits

Thermo Scientific™ Pierce™ Magnetic IP/Co-IP Kits are optimized to isolate protein complexes from biological samples. Each kit contains all the required buffers and beads validated to deliver the best results. Three versions of the kit are available to perform a classic IP, crosslink IP or direct IP.

Kit Highlights

- Compatible with any antibody
- Faster immunoprecipitations (IPs) for less background
- Easily capture transient protein complexes
- No antibody contamination in your eluted sample
- Simple handling with no sample loss
- Validated for automated protocols using KingFisher Instruments

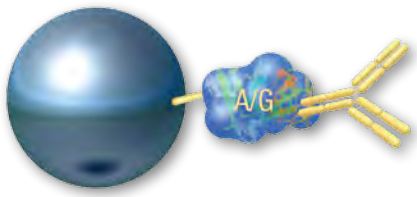


The Thermo Scientific™ Pierce™ Classic Magnetic IP/Co-IP Kit

uses high binding capacity Pierce Magnetic Protein A/G Beads to deliver clean and consistent co-immunoprecipitations (co-IP) with any common antibody. Antibodies are not linked to the resin and will co-elute with your antigen.

Select this version:

- For the highest antigen yield
- If antibody contamination is not a concern



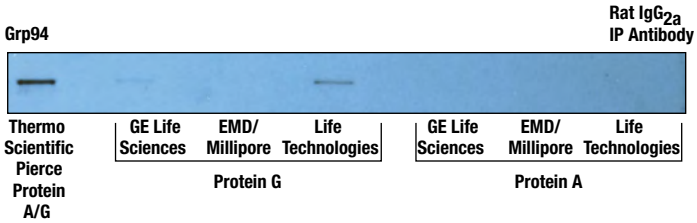


Figure 16. The Thermo Scientific Pierce Classic Magnetic IP/Co-IP Kit immunoprecipitates Grp94 with higher yield than other Protein A and Protein G beads. MOPC (mouse myeloma) cells were lysed in RIPA buffer and 0.75mg of lysate was incubated with Grp94 antibody (rat IgG_{2a}) overnight at 4°C. Using the KingFisher Flex Instrument, 50µL of Pierce Protein A/G Magnetic Beads and 50µL each of Protein A and Protein G beads from Life Technologies, EMD/ Millipore and GE Life Sciences were added to a 96 deep-well plate. The eluates were resolved by SDS-PAGE and analyzed by Western blot for Grp94.

Ordering Information

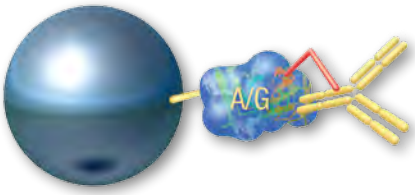
Product #	Description	Pkg. Size
88804	**Pierce Classic Magnetic IP/Co-IP Kit** *Sufficient for: 40 IP reactions using 25µL of beads* Contains: Pierce Protein A/G Magnetic Beads, 1mL Pierce IP Lysis/Wash Buffer, 2 x 50mL Lane Marker Sample Buffer (5X), 5mL Elution Buffer, 5mL Neutralization Buffer, 0.5mL	40-rxn kit

The Thermo Scientific™ Pierce™ Crosslink Magnetic IP/Co-IP Kit

uses crosslinkers to immobilize your primary antibody to Protein A/G. This prevents antibody contamination in your eluted sample and eliminates antibody interference in Western blot and mass spec applications.

Select this version:

- To eliminate antibody contamination that interferes with downstream detection
- To properly orient your antibody



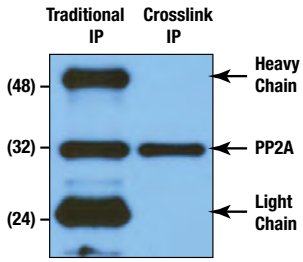


Figure 17. The Thermo Scientific Pierce Crosslink Magnetic IP/Co-IP Kit immunoprecipitates PP2A without antibody contamination and with negligible background. PP2A antibody (5µg) was coupled to Pierce Protein A/G Magnetic Beads without DSS crosslinking (traditional IP) and with DSS crosslinking (crosslink IP). The beads were incubated with 0.5mg of A549 cell lysate for one hour at room temperature on the KingFisher Flex Instrument. PP2A was eluted from the beads with elution buffer for five minutes at room temperature and then neutralized with neutralization buffer. The eluates, antibody control (Ab) and flow-through (FT) were resolved by SDS-PAGE and analyzed by Western blot for PP2A. The antibody-crosslinked Pierce Protein A/G Magnetic Beads effectively immunoprecipitated PP2A without antibody contamination whereas the traditional IP method resulted in significant antibody contamination in the eluate.

Ordering Information

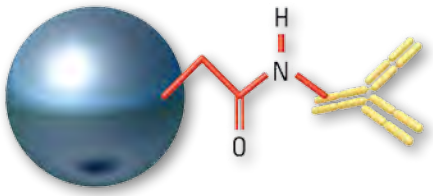
Product #	Description	Pkg. Size
88805	**Pierce Crosslink Magnetic IP/Co-IP Kit** *Sufficient for: 40 IP reactions using 25µL of beads* Contains: Pierce Protein A/G Magnetic Beads, 1mL IP Lysis/Wash Buffer, 2 x 50mL Coupling Buffer (20X), 25mL DSS Crosslinker, 8 x 2mg Lane Marker Sample Buffer (5X), 5mL Elution Buffer, 10mL Neutralization Buffer, 1mL Lane Marker Sample Buffer (5X), 5mL	40-rxn kit

The Thermo Scientific™ Pierce™ Direct Magnetic IP/Co-IP Kit

uses Pierce NHS-Activated Magnetic Beads to immobilize your primary antibody directly to the bead surface. This method is independent of antibody species and prevents antibody contamination in your eluted sample.

Select this version:

- For non-traditional antibodies that do not bind Protein A or Protein G
- To eliminate antibody contamination



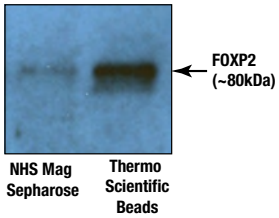


Figure 18. Better immunoprecipitation results with Thermo Scientific Pierce NHS-Activated Magnetic Beads. Anti-FOXP2 antibody (5µg) was coupled to 25µL of Pierce NHS-Activated Magnetic Beads and an equivalent amount of NHS Mag Sepharose (GE Life Sciences). The two sets of prepared beads were then used to immunoprecipitate FOXP2 from 0.5mg aliquots of the same 293T (human epithelial kidney) cell lysate. The eluates were resolved by SDS-PAGE and analyzed by Western blot for FOXP2.

Ordering Information

Product #	Description	Pkg. Size
88828	**Pierce Direct Magnetic IP/Co-IP Kit** *Sufficient for: 40 IP reactions using 25µL of beads* Contains: Pierce NHS-Activated Magnetic Beads, 1mL IP Lysis/Wash Buffer, 2 x 50mL Elution Buffer, pH 2.0, 5mL Lane Marker Sample Buffer, Non-reducing, (5X), 5mL Neutralization Buffer, pH 8.5, 0.5mL 0.67M Borate Buffer, 1mL BupH™ Borate Buffer Pack, 1 pack Quenching Buffer, 25mL	40-rxn kit

select an easy-to-use **validated kit**

Magnetic Anti-HA IP Kit

The Thermo Scientific™ Pierce™ HA-Tag Magnetic IP/Co-IP Kit provides a simple and fast method to study protein interactions. The high affinity anti-HA antibody-coupled magnetic beads enables immunoprecipitation (IP) of HA-tagged proteins or co-immunoprecipitation (co-IP) of their interacting partners without antibody contamination.

Highlights

- **Specific** – immunoprecipitate only HA-tagged proteins and their interactors
- **Validated** – IP/co-IP kit includes a positive control lysate
- **Robust** – compatible with common tissue culture cell lysates
- **Convenient and easy** – complete kit includes all necessary reagents to perform 40 reactions

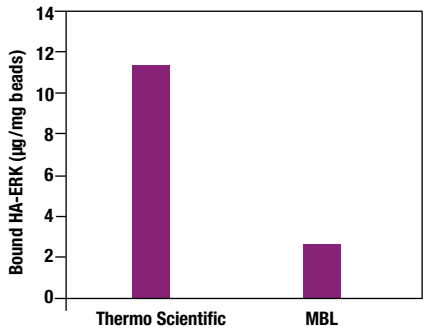


Figure 19. Significantly higher binding capacity with Thermo Scientific Pierce Anti-HA Magnetic Beads. HA-tagged protein (HA-ERK-GST, 400µg in PBS) was incubated with 100µL each of Thermo Scientific™ Pierce™ Anti-HA Magnetic Beads or MBL Anti-HA Magnetic Beads for one hour at room temperature. Bound HA-tagged protein was measured using the Pierce BCA Assay. Pierce Anti-HA Magnetic Beads pulled down more than four times as much protein as the equivalent amount of MBL Anti-HA Magnetic Beads.

Table 6. Properties of Thermo Scientific Pierce Anti-HA Magnetic Beads.

Composition	Magnetite-coated polymeric beads blocked and covalently coated with a mouse monoclonal IgG1 anti-HA antibody
Mean Diameter	1µm (nominal)
Density	2.0g/cm^3
Bead Concentration	10mg/mL in water with sodium azide
Binding Capacity	> 10µg of HA-tagged protein/mg beads (70kDA)

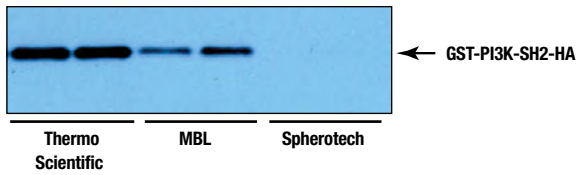
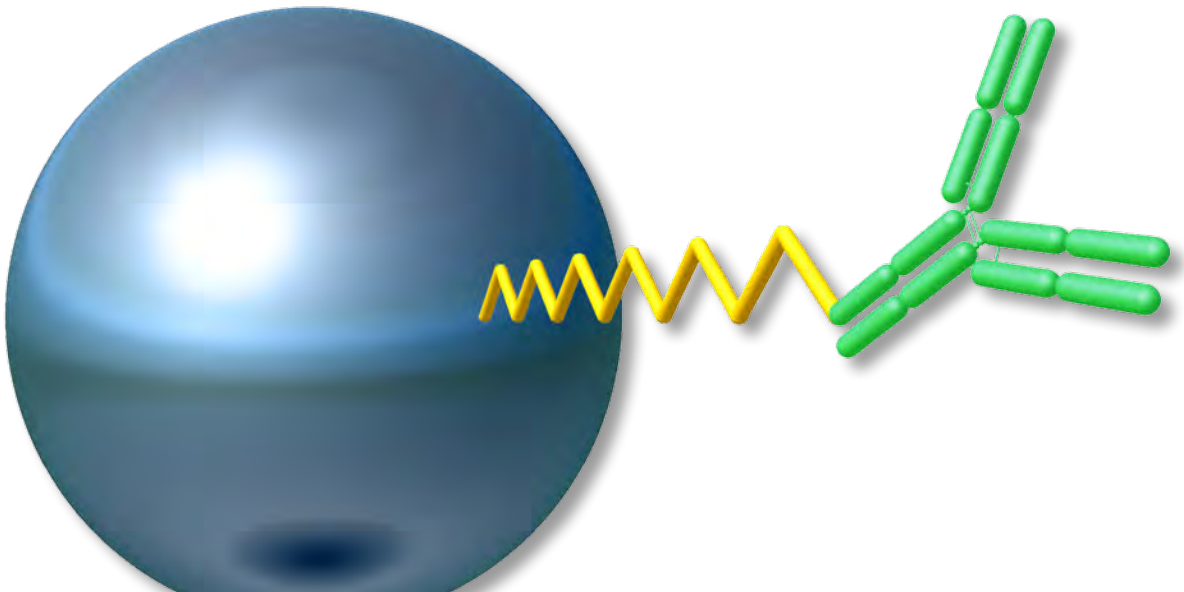


Figure 20. Better Immunoprecipitation results from *E. Coli* lysates. Using a KingFisher Flex Instrument with 96 deep-well plates, 25µL each of Pierce Anti-HA Magnetic Beads, Anti-HA-tag Magnetic Beads (MBL International Corp.) and SPHERO™ Rabbit Anti-HA Magnetic Beads (Spherotech Inc.) were used to immunoprecipitate GST-PI3K-SH2-HA from 50µg of *E. coli* lysate in duplicate. Captured protein was eluted with 0.1M glycine, pH 2.0, and then resolved by SDS-PAGE and analyzed by Western blot for the HA-tagged protein.



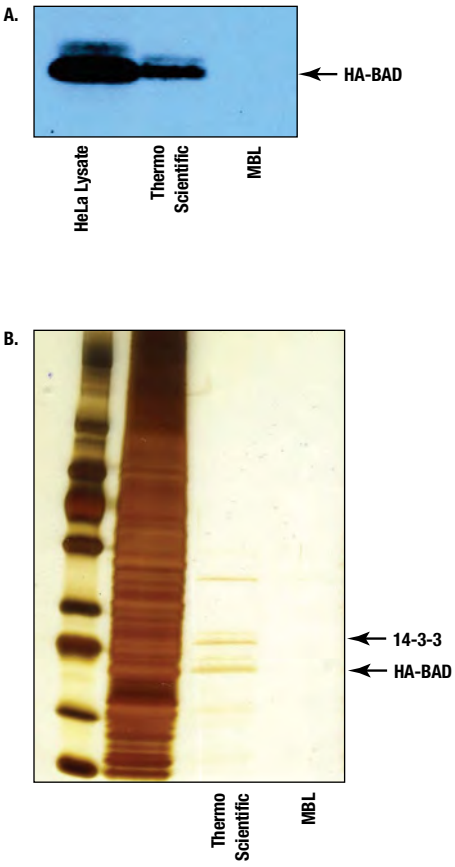


Figure 21. Better immunoprecipitation of protein expressed *in vitro*. Using the KingFisher Flex Instrument, 25µL each of Pierce Anti-HA Magnetic Beads and Anti-HA-tag Magnetic Beads (MBL International Corp.) were added to 96 deep-well plates. The beads were incubated for one hour with HA-tagged BAD protein, expressed using the Thermo Scientific™ 1-Step High-Yield *in vitro* Translation Kit. After one hour at room temperature, samples were washed twice in phosphate-buffered saline containing 0.05% Tween-20, washed once in water and then eluted in 0.1M glycine, pH 2.0. Samples were resolved by SDS-PAGE and analyzed by Western blot for HA (**Panel A**) and by silver stain for nonspecific binding (**Panel B**). HA-BAD fusion protein yield was highest with Pierce Anti-HA Magnetic Beads compared to other anti-HA beads. Nonspecific binding was negligible and there was co-immunoprecipitation of the protein 14-3-3.

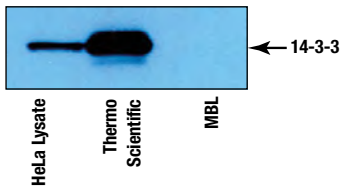


Figure 22. Better co-IP results with Thermo Scientific Pierce Anti-HA Magnetic Beads. Serine phosphorylation of BAD is associated with 14-3-3 binding and inhibition of BAD-induced cell death. Using a magnetic stand, 50µL each of Pierce Anti-HA Magnetic Beads and Anti-HA-tag Magnetic Beads (MBL International Corp.) were added to microcentrifuge tubes. The beads were incubated for one hour at room temperature with HA-tagged BAD expressed in the 1-Step Human High-Yield IVT Kit. After incubation, the beads were washed twice in phosphate-buffered saline containing 0.05% Tween-20, washed once in water and then eluted in 30% acetonitrile/0.5% formic acid. Samples were dried down in a speedvac and brought back up in 50µL of reducing SDS-PAGE samples buffer. One half of the reconstituted eluate was resolved by SDS-PAGE and analyzed by Western blot for 14-3-3. Pierce Anti-HA Magnetic Beads were found to have higher yield of 14-3-3 than the other anti-HA beads. Nonspecific binding was negligible.

Ordering Information

Product #	Description	Pkg. Size
88836	**Pierce Anti-HA Magnetic Beads**	1mL
88837	**Pierce Anti-HA Magnetic Beads**	5mL
88838	**Pierce HA-Tag Magnetic IP/Co-IP Kit** *Sufficient For: 40 IP reactions using 25µL of anti-HA magnetic beads* Kit Contents: HA-tagged Positive Control (Product # 26180X), 500uL Application Set (Product # 88838X): Pierce Anti-HA Magnetic Beads, 0.65mL Pierce IP Lysis/Wash Buffer, 2 × 50mL, pH 7.4 Lane Marker Sample Buffer, Non-reducing, (5X), 5mL, pH 6.8 Elution Buffer, 5mL, pH 2.0 Neutralization Buffer, 1mL, pH 8.5	40-rxn kit

select an easy-to-use **validated kit**

Magnetic ChIP Kit

The Thermo Scientific™ Pierce™ Magnetic ChIP Kit provides a simple, fast and reproducible method to perform chromatin immunoprecipitation (ChIP) assays to capture a snapshot of specific protein-DNA interactions as they occur in living cells and then quantitate the interactions using PCR.

The Pierce Magnetic ChIP Kit contains sufficient reagents to perform 30 ChIP assays with appropriate controls using an optimized protocol. The blocked Pierce Protein A/G Magnetic beads used in this kit provide high binding capacity, low nonspecific background and flexibility of antibody species. These beads can be used manually with a magnetic stand as well as with automated platforms such as KingFisher Instruments. This kit provides reagents and a method to capture protein-DNA interactions *in vivo* allowing relative protein binding events to be monitored under different conditions and/or treatments. ChIP-validated and quality-guaranteed antibodies are also available for use with the Pierce Magnetic ChIP Kit.

Highlights

- **Simple and fast** – obtain purified DNA ready for PCR in about eight hours
- **Efficient and reproducible** – micrococcal nuclease digestion and nuclear lysis are highly optimized
- **Sensitive** – obtain results with as little as $1x10^4$ cells
- **Low nonspecific background** – Pierce Protein A/G Magnetic beads are blocked in a non-DNA-containing reagent to minimize background
- **Complete** – optimized positive control reagents are included: RNA polymerase II antibody and GAPDH promoter PCR primers

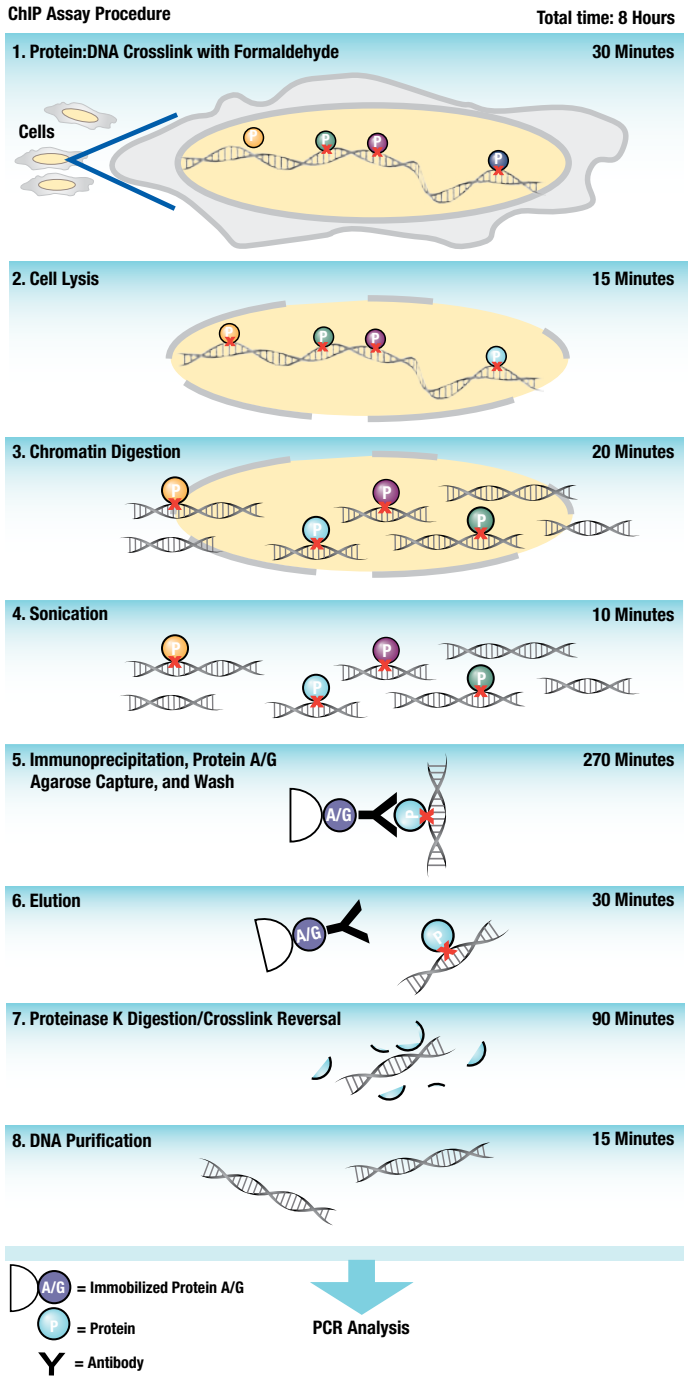
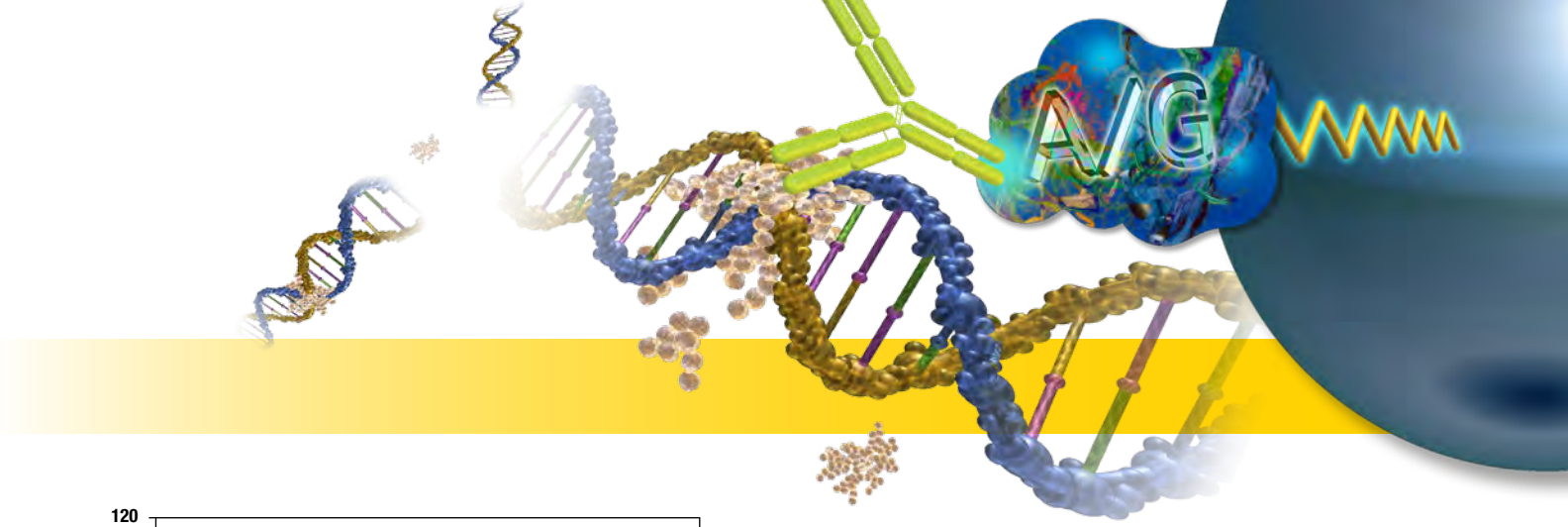


Figure 23. Overview of the Thermo Scientific Pierce Magnetic ChIP Kit protocol. The Pierce Magnetic ChIP assay protocol and reagents provide an optimized system for performing chromatin crosslinking, cell lysis, IP and target protein recovery about eight hours.



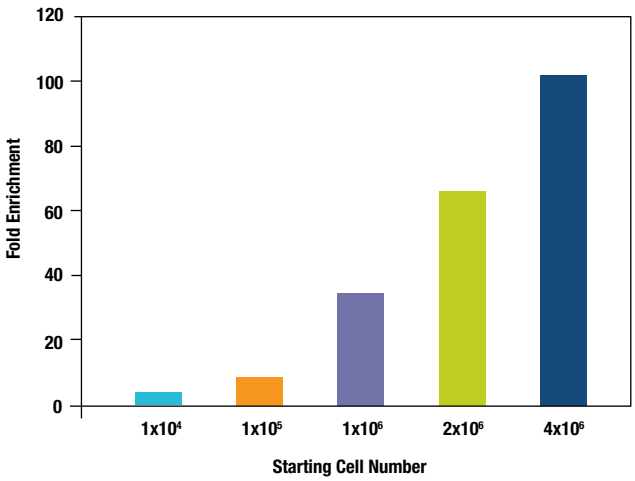


Figure 24. The Thermo Scientific Pierce Magnetic ChIP Kit has a broad range of sensitivity. A431 lung carcinoma cells were crosslinked using a final concentration of 1% formaldehyde for 10 minutes. ChIP assays were performed with the Pierce Magnetic ChIP Kit to determine binding of RNA polymerase II to the proximal GAPDH promoter. Quantitative real-time PCR data was obtained with a Bio-Rad iQ5™ Thermocycler. Each column represents the fold enrichment of the RNA polymerase II over the normal rabbit IgG using the noted starting cell number (i.e., chromatin from that number of cells).

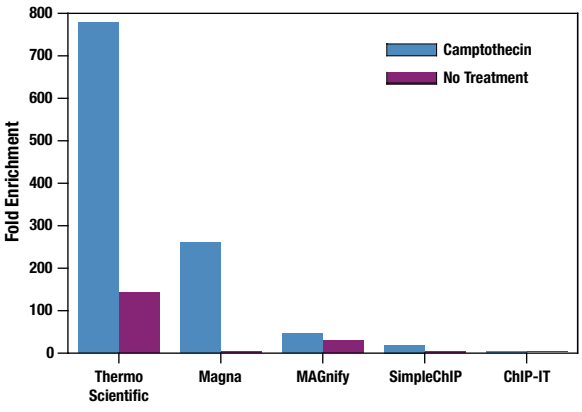


Figure 25. Greater fold enrichment than other kits. LNcaP prostate carcinoma cells were cultured in RPMI-1640 containing 10% FBS for 24 hours. Half of the cultures plated were treated for 16 hours with 5µM camptothecin, a drug that inhibits DNA topoisomerase I. Crosslinking was achieved using a final concentration of 1% formaldehyde in the media for 10 minutes. ChIP assays were performed according to the manufacturers' protocols to determine binding of p53 to a 1.5-kb region of the CDKN1A (p21) promoter. Quantitive real-time PCR data was obtained with a Bio-Rad iQ5 Thermocycler. The Pierce Magnetic ChIP Kit has been optimized to isolate even large DNA fragments.

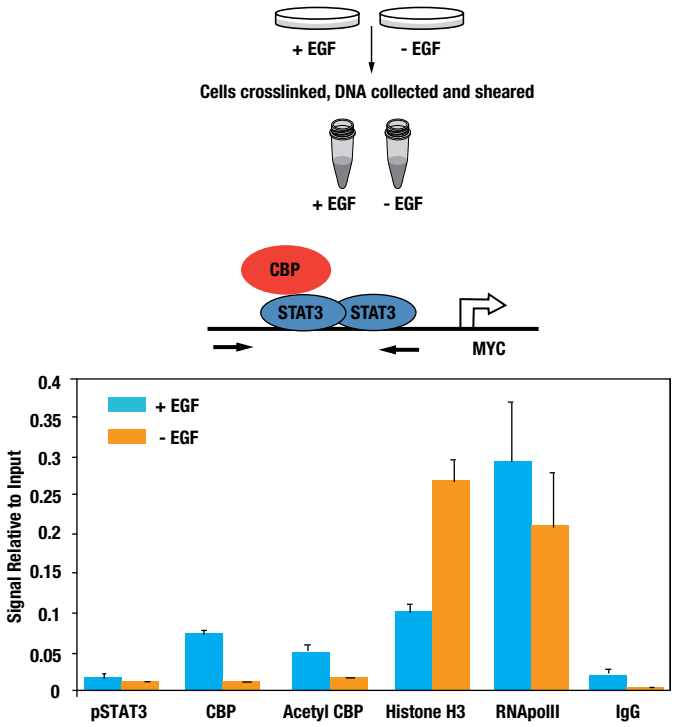


Figure 26. Profiling multiple transcription factors binding to the MYC promoter using the Thermo Scientific Pierce Magnetic ChIP Kit . A431 lung carcinoma cells were cultured in DMEM containing 10% FBS for 24 hours. Following a 24-hour serum withdrawal, half of the cultures plated were treated with 100ng/mL EGF for 10 minutes. Crosslinking was achieved using a final concentration of 1% formaldehyde in the media for 10 minutes. ChIP assays were performed with the Pierce Magnetic ChIP Kit to determine binding of phosphorylated-STAT3, CBP, acetylated-CBP, histone H3, and RNA polymerase II to the proximal MYC promoter. Primary antibody amounts were determined empirically. Quantitive real-time PCR data was obtained with a Bio-Rad iQ5 Thermocycler. Graph represents the signal relative to the total input of chromatin.

Ordering Information

Product #	Description	Pkg. Size
26157	**Pierce Magnetic ChIP Kit** *Sufficient reagents to perform 30 ChIP Reactions*	30-rxn kit
26162	**ChIP-grade Protein A/G Magnetic Beads** *Formulation: Magnetite- and protein-coated polymer beads at 10mg/mL in water with sodium azide.* *Sufficient For: Use in approx. 250 typical ChIP assays*	5mL

select an easy-to-use **validated kit**

Magnetic RNA-Protein Pull-Down Kit

The Thermo Scientific™ Pierce™ Magnetic RNA-Protein Pull-Down Kit provides researchers with a streamlined, robust method for enrichment and identification of RNA binding proteins. This method uses RNA probes labeled at the 3'- end with desthiobiotin and magnetic streptavidin particles. The complete kit contains sufficient reagents for 20 RNA-labeling reactions and 20 RNA-protein pull-down assays. Both synthetic RNA or *in vitro* transcribed RNA can be labeled with desthiobiotin. RNA-binding proteins are then enriched from cellular/tissue lysates or from *in vitro* translated protein preps. RNA-binding proteins are detected using Western blotting or mass spectrometry (MS).

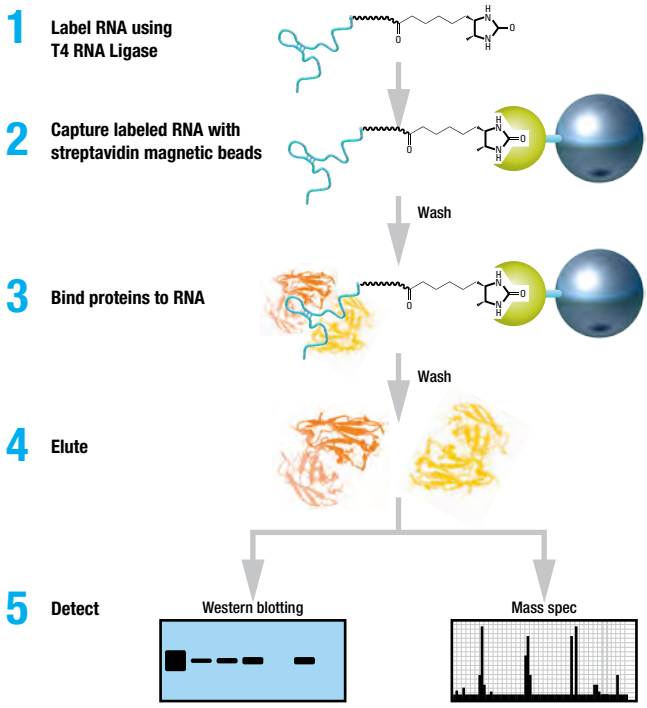


Figure 27. Easy RNA labeling and interaction analysis. RNA probes are first labeled at the 3' end with desthiobiotin using T4 RNA Ligase. RNA probes are then immobilized onto magnetic streptavidin particles and incubated with protein from cell lysates or *in vitro* translation preps. RNA-binding proteins are eluted and detected by Western blotting or mass spec.

Highlights

- **Direct** – capture ribonucleoprotein complexes directly from cell lysates
- **Easy to use** – magnetic beads enable easy processing for multiple samples
- **Flexible** – enrich RNA binding proteins from cell/tissue lysates or *in vitro* translated protein preps
- **Clean** – magnetic format yields low background
- **Specific** – perform RNA mutations to map interaction sites
- **Complete** – contains both labeling and enrichment modules with buffers necessary for assay; positive control RNA, negative control RNA and anti-RBP antibody included

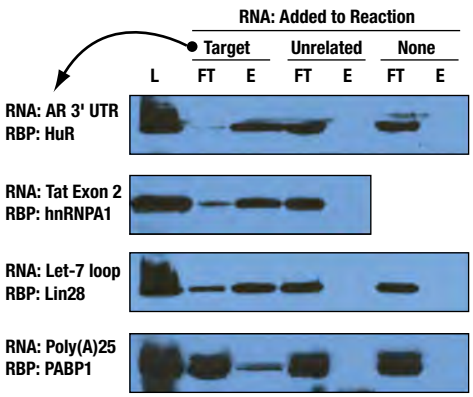
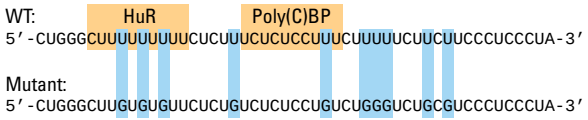


Figure 28. End-labeled RNA enriches specific target binding proteins. RNA binding proteins (RBP) of the AR 3' UTR control system (top panel) and three experimental systems were enriched according to kit procedure. L = lysate; FT = flow-through; E = elute.

Incubation of A431 lysate with labeled AR UTR RNA (Target) enriches HuR RBP, while incubation with negative control poly(A) RNA (Unrelated) or beads only (None) does not (compare elution lanes). The same pattern results with the experimental systems, confirming the proper function of the kit. Samples were normalized by volume, and bands were detected using Thermo Scientific™ SuperSignal™ West Pico Substrate by a 2-minute exposure to film. Target RNA sequences were as follows:

Androgen Receptor 3' UTR (Kit Control System):
5'-CUGGGCUUUUUUUUUCUCUUUCUCUCCUUUCUUUUUCUUCUUCCCUCCCUA-3'
Tat Exon 2:
5'-UUACUCAACAGAGGAGAGCAAGAAAUGGAGCCAGUAGAUCCUAGACUAGAGCCCUGG-3'
Let-7 loop:
5'-CAGUUUGAGGGUCUAUGAUACCACCGGUACAAGAUAACUG-3'
Poly(A)25:
5'-AAAAAAAAAAAAAAAAAAAAAAAAA-3'

A. Androgen Receptor 3' UTR RNA Sequence



B. Magnetic Pull-Down Results

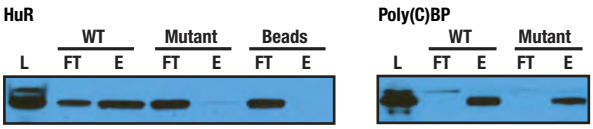


Figure 29. Androgen receptor 3'-UTR RNA specifically pulls-down HuR. Wild-type and mutant AR 3'-UTR RNA (50pmol) were labeled using cytidine bisphosphate desthiobiotin and T4 RNA ligase. Labeled RNA was captured using 50µL of streptavidin magnetic beads in RNA capture buffer for 30 minutes at room temperature. Beads were washed twice in 20mM Tris (pH 7.5), once in Protein-RNA binding buffer, and 40µg of A431 extract was added. Samples were incubated 45 minutes at 4°C, washed three times with wash buffer and eluted after 15 minutes of incubation at 37°C with elution buffer. RNA pull-down specificity was assessed by Western blotting. Samples were normalized by volume and bands were detected using SuperSignal West Pico Substrate. Exposure time = 2 minutes. L – lysate load; FT – flow-through, E – elute. PCBP1 antibody (1:1000, Genway Biotech #GWB-38F6F3).

MS Peptide Identification

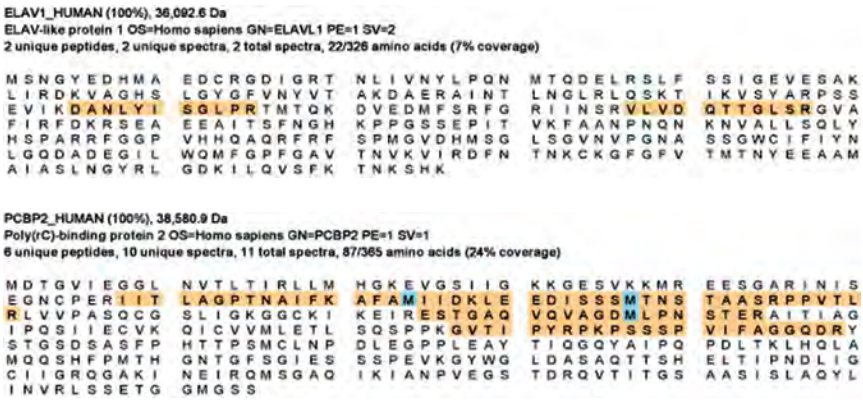


Figure 30. RNA:protein interactions enriched by the Thermo Scientific Pierce Magnetic RNA-Protein Pull-Down Kit can be detected via mass spectrometry. Proteins bound to the wild-type or mutant versions of the androgen 3'-UTR RNA sequence were enriched and analyzed by mass spectrometry. Samples enriched using the Pierce Magnetic RNA-Protein Pulldown Kit were prepared using the Thermo Scientific™ Pierce™ In-Gel Tryptic Digestion Kit. Each sample was diluted to 50µL with 0.1% TFA and 5µL was analyzed by LC-MS using a Thermo Scientific™ Velos Pro Mass Spectrometer with a top-20 data dependent method. Peptides were separated using a 5-40% gradient (buffer A: 0.1% FA; buffer B: 0.1% FA/100% acetonitrile) over 40 minutes using a ProteoPep™ II C18 15 cm column (New Objective) and ionized using a Thermo Scientific™ Nanospray Flex Ion Source instrument. MS spectra were searched using Mascot™ software against a mammalian SwisProt database using carbamidomethyl as a fixed cysteine modification and methionine oxidation as a variable modification. Scaffold Q+ software was used to analyze the search results using 99% peptide confidence and 90% protein confidence levels with a minimum of two peptides identified per protein. Protein sequence and peptide coverage are indicated in yellow. The spectral counts reveal that RNA mutation of the HuR binding site reduced HuR binding, while poly(rC)-binding protein1 and protein2 showed no significant decrease in binding.

Label-free MS Quantitation Using Spectral Counts

Protein	Accession Number	Mutant	Wild-Type
HuR (ELAV-like protein 1)	ELAV1_HUMAN	0	2
Poly(rC)-binding protein 2	PCBP2_HUMAN	7	10
Poly(rC)-binding protein 1	PCBP1_HUMAN	2	3

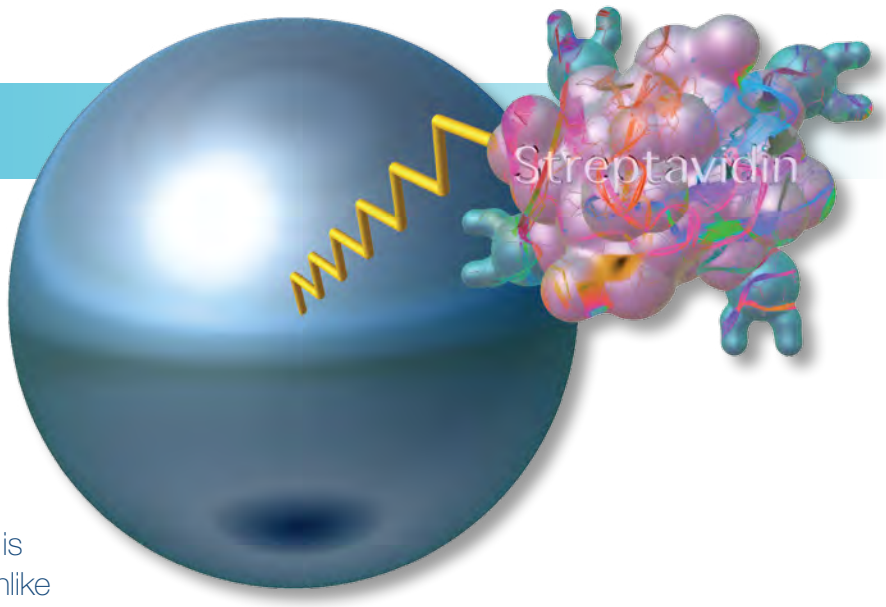
Ordering Information

Product #	Description		Pkg. Size
20163	**Pierce RNA 3' End Desthiobiotinylation Kit**		20-rxn kit
	Sufficient For: 20 desthiobiotinylation reactions using 50pmol of RNA each		
	Kit Contents:	Desthiobiotinylated Cytidine Bisphosphate	40µL
		T4 RNA Ligase	40µL
		T4 RNA Ligase Reaction buffer (10X)	100µL
		Non-labeled RNA Control	100µL
		Biotinylated IRE RNA Control	35µL
		RNase Inhibitor	2 x 10µL
		DMSO	200µL
		PEG (30%)	300µL
		Glycogen,	20µL
		Nuclease-free Water	1.5mL

20164	**Pierce Magnetic RNA-Protein Pull-Down Kit**		20-rxn kit
	Sufficient For: 20 RNA-protein complex pull-down reactions		
	Kit Contents:	RBP Enrichment Module (Part No. 20164Y, store at 4°C):	
		Pierce Nucleic Acid-Compatible Streptavidin Magnetic Beads	1mL
		RNA Capture Buffer (1X)	10mL
		Tris (20mM, pH 7.5)	5mL
		Protein-RNA Binding Buffer (10X)	1mL
		Wash Buffer (1X)	10mL
		Biotin Elution Buffer	1.5mL
		HuR Monoclonal Antibody (Mouse)	50µL
		RNA Controls (Part No. 20164Z, store at -20°C):	
		Positive RNA Control (AR RNA)	250pmol
		Negative RNA Control (polyA25 RNA)	250pmol
		Pierce RNA 3' End Desthiobiotinylation Kit (Part No. 20163, store at -20°C) (shipped separately on dry ice)	

perform **high-capacity** protein purification

Magnetic Biotin Pull-Down



Thermo Scientific™ Pierce™ Streptavidin Magnetic Beads provide easy affinity purification of biotin-labeled target molecules without columns or centrifugation. Pierce Streptavidin Magnetic Beads use a recombinant form of streptavidin with a mass of 53kDa and a near-neutral isoelectric point (pI). The protein is a tetramer having four biotin-binding sites. Unlike avidin, streptavidin has no carbohydrate groups, resulting in low nonspecific binding. The high-affinity interaction between streptavidin and biotin cannot be dissociated efficiently except under very harsh conditions, such as boiling in sample loading buffer for SDS-PAGE or 8 M guanidine•HCl, pH 1.5. Consequently, it is often possible to elute binding partners in an interaction complex without also eluting the biotinylated component.

Highlights

- **Stable immobilization chemistry** – streptavidin is immobilized using leach-resistant chemistry
- **High capacity** – superior-quality beads with high binding capacity provide rapid and efficient biomolecule purification from complex samples
- **Low nonspecific binding** – stable, pre-blocked beads provide clean purification products that are compatible with mass spectrometry analysis
- **Superior performance** – binding capacity is nearly three times higher than beads from other suppliers, allowing the use of smaller samples per experiment

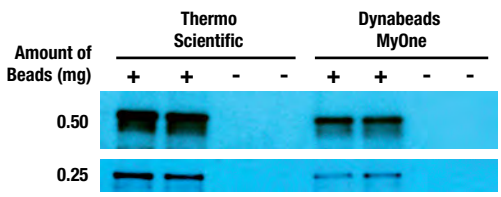


Figure 31. Better immunoprecipitation results with Thermo Scientific Pierce Streptavidin Magnetic Beads. MOPC cell lysate (0.75mg per sample) was incubated overnight at 4°C with and without 10µg biotinylated Grp94 antibody. Pierce Streptavidin Magnetic Beads and Dynabeads MyOne™ Streptavidin T1 Beads (Life Technologies) were added to a 96 deep-well plate (0.5mg or 0.25mg per well). Eluates were resolved by SDS-PAGE and analyzed by Western blot with anti-Grp94 antibody. About 0.25mg of Pierce Streptavidin Magnetic Beads gave the same yield as 0.5mg of Dynabeads MyOne T1 Beads.

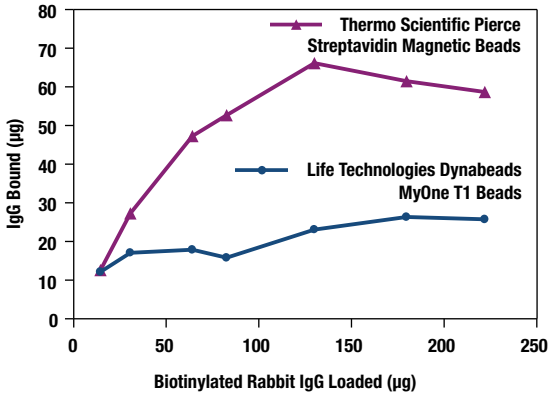


Figure 32. Higher binding capacity with Thermo Scientific Pierce Streptavidin Magnetic Beads. Pierce Streptavidin Magnetic Beads and Life Technologies Dynabeads MyOne Streptavidin T1 Beads were added to a 96 deep-well plate (1mg beads per well). Using the KingFisher 96 Instrument, the beads were washed with phosphate-buffered saline containing 0.05% Tween-20. The beads were then incubated for one hour with varying amounts of biotinylated rabbit IgG (20-225µg).

Ordering Information

Product #	Description	Pkg. Size
88816	**Pierce Streptavidin Magnetic Beads** *Sufficient for: Binding approx. 55µg biotinylated rabbit IgG per mg of beads (approx. 3500 pmol biotinylated fluorescein per mg of beads)*	1mL
88817	**Pierce Streptavidin Magnetic Beads** *Sufficient for: Binding approx. 55µg biotinylated rabbit IgG per mg of beads (approx. 3500 pmol biotinylated fluorescein per mg of beads)*	5mL

Magnetic GST-Tagged Protein Purification

Thermo Scientific™ Pierce™ Glutathione Magnetic Beads provide a simple, rapid and reliable method for the purification of glutathione S-transferase (GST) fusion proteins from crude cell lysate prepared from bacteria, yeast or mammalian cells. These beads can be used to isolate GST-tagged proteins or perform pull-down assays using GST-tagged proteins as bait.

Highlights

- **High binding** – 5-10mg GST/mL settled beads
- **Stable affinity ligand** – glutathione is covalently immobilized to particles, ensuring clean, leach-resistant purification products
- **High capacity** – binding capacity is sufficient for both routine and demanding magnetic separation procedures

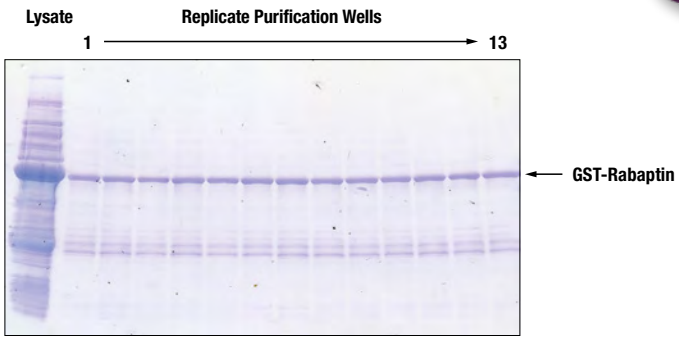
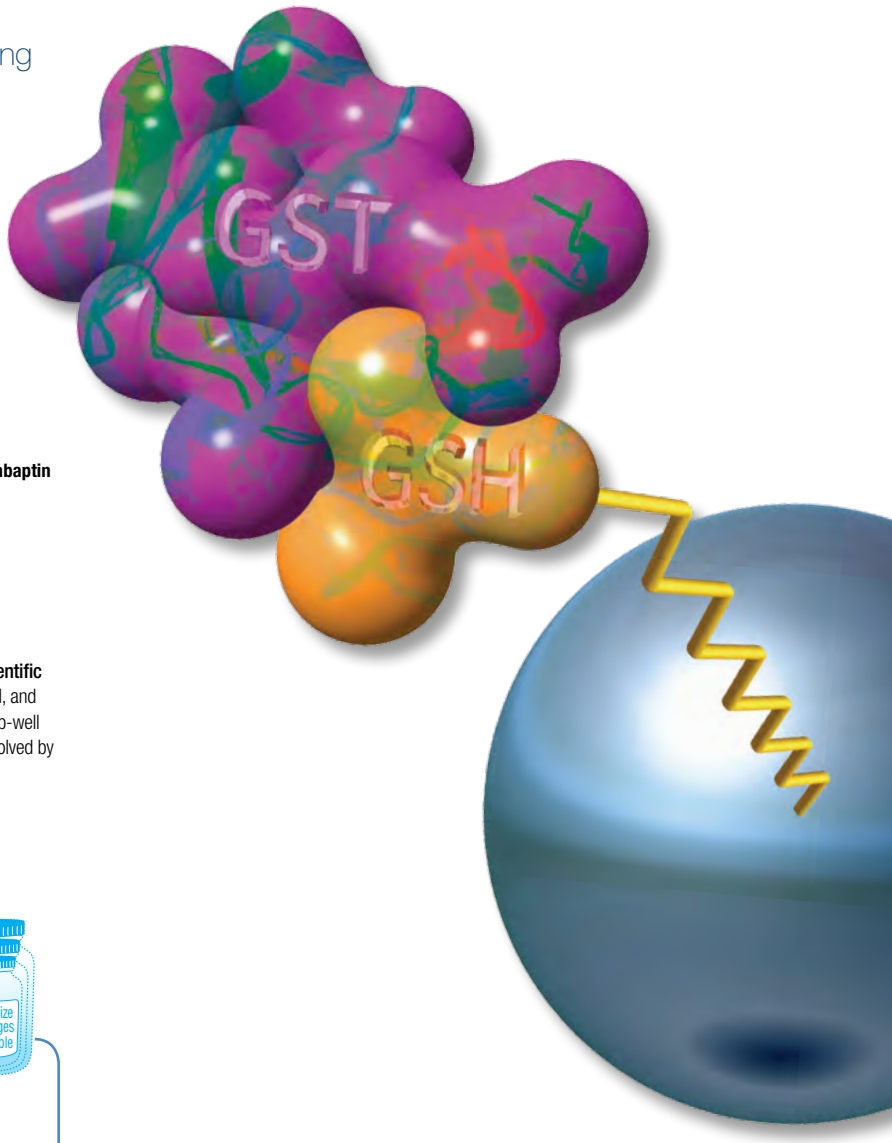


Figure 33. High-performance purification of a GST fusion protein using Thermo Scientific Pierce Glutathione Magnetic Beads. Bacterial cells expressing GST-Rabaptin were lysed, and replicate aliquots were processed with the Pierce Glutathione Magnetic Beads in a 96 deep-well plate using a KingFisher 96 Instrument. Eluates were boiled in reducing sample buffer, resolved by SDS-PAGE and stained with coomassie dye. Purity and reproducibility were excellent.



Ordering Information

Product #	Description	Pkg. Size
88821	**Pierce Glutathione Magnetic Beads** *Sufficient for: Binding 5 to 10mg GST per mL of beads*	4mL
88822	**Pierce Glutathione Magnetic Beads** *Sufficient for: Binding 5 to 10mg GST per mL of beads*	20mL

perform **high-capacity** protein purification

Magnetic His-Tagged Protein Purification

Thermo Scientific™ HisPur™ Ni-NTA Magnetic Beads are high-capacity Nickel-IMAC beads for affinity purification of His-tagged fusion proteins in manual or automated formats. The blocked magnetic bead surface is derivatized with the nitrilotriacetic acid (NTA) chelation moiety and loaded with divalent nickel ions (Ni^{2+}). The immobilized metal affinity chromatography (IMAC) beads provide high binding capacity with very low background. The HisPur Ni-NTA Magnetic Beads can be used both manually with a magnetic stand as well as with automated platforms such as the KingFisher Instruments for high-throughput needs.

Highlights

- **High capacity** – equivalent or higher binding capacity than Ni-NTA magnetic beads from other suppliers
- **Low nonspecific binding** – the bead surface is pre-blocked and the protocol provides optimized buffers for purification
- **Fast** – protocol is completed in less than one hour
- **Scalable** – process microliter to milliliter sample volumes
- **Versatile** – purify proteins using native or denaturing conditions
- **Reagent compatible** – can be used with common cell lysis reagents and a variety of buffer additives
- **Multiple formats** – protein coupling to the beads and downstream applications can be performed both manually and on an automated platform (e.g., KingFisher Instruments)

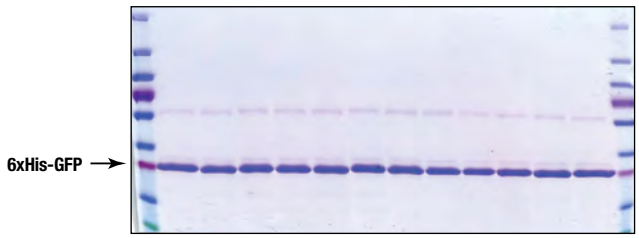
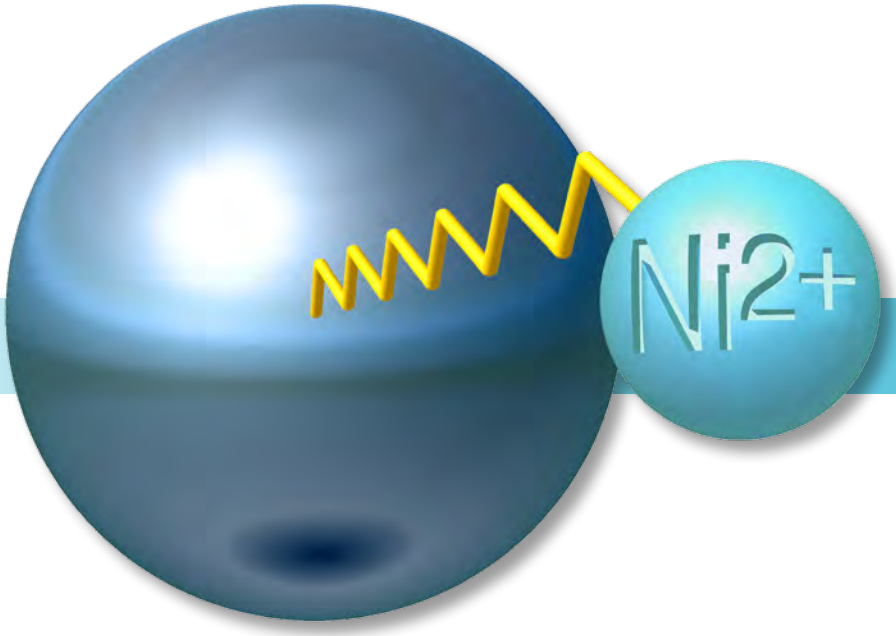


Figure 34. Thermo Scientific HisPur Ni-NTA Magnetic resin delivers consistent yield. His-tag protein purification was performed in a 96-well plate using a KingFisher Flex Instrument. In each well, 100µg of *E. coli* lysate expressing 6xHis-GFP protein was added to 0.5mg of Thermo Scientific™ Pierce™ Magnetic Ni-NTA Resin. Eluted protein was analyzed by SDS-PAGE stained with Imperial Protein Stain to determine well-to-well consistency in protein recovery. The variance between samples is measured at less than 15%.



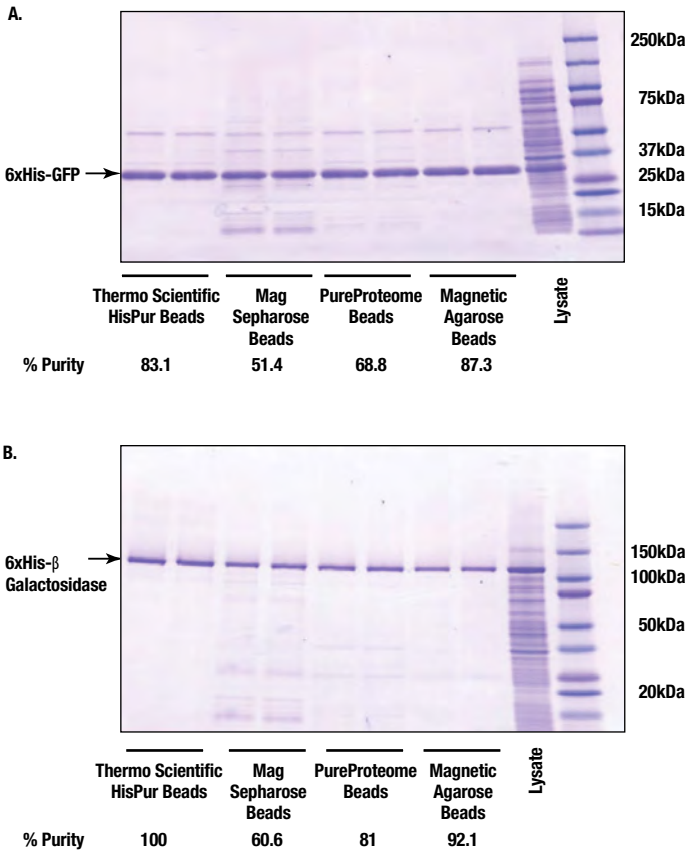
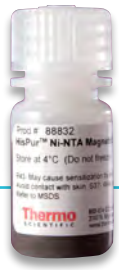


Figure 35. Superior performance of Thermo Scientific HisPur Ni-NTA Magnetic Beads. Bacterial lysate (100µL total protein) containing over-expressed 6xHis-GFP **(Panel A)** or over-expressed 6xHis-β Galactosidase **(Panel B)** was applied to 0.5mg of HisPur Ni-NTA Magnetic Beads, Mag Sepharose (GE Life Sciences), PureProteome (EMD/Millipore) or Magnetic Agarose (Qiagen) Ni-NTA beads. All samples were run in duplicate, and the beads were processed using buffers recommended by the manufacturers. For the HisPur Ni-NTA Magnetic Beads, the amount of imidazole in the equilibration, wash and elution buffers was 30mM, 50mM and 150mM, respectively. All three buffers contained 100mM sodium phosphate and 600mM sodium chloride. Binding was performed with all samples for 30 minutes. The beads were collected on a magnetic stand and the flow-throughs were saved for analysis. Eluates were resolved on an SDS-PAGE gel and stained with Imperial Stain. For purification of 6xHis-GFP, comparable yields and purity were observed for HisPur Ni-NTA and Qiagen Ni-NTA Magnetic Beads. HisPur Ni-NTA Magnetic Beads showed higher yield and purity than the Qiagen Magnetic Agarose Ni-NTA beads in the purification of 6xHis-β Galactosidase. The Mag Sepharose and PureProteome Ni-NTA Magnetic beads gave lower purity and lower yield than HisPur Ni-NTA Magnetic Beads in both purifications. Purity analyses were performed on a Thermo Scientific™ MYECL™ Imager with Thermo Scientific™ MYIMAGEANALYSIS™ Software. Purity was determined by measuring the ratio of the background-corrected 6xHis-tagged protein band of interest to the sum of all bands in a given lane.

Table 8. Characteristics of Thermo Scientific HisPur Ni-NTA Magnetic Beads.

Composition	Ni^{2+} loaded on nitrilotriacetic acid that has been covalently coupled to the beads
Mean Diameter	1µm (nominal)
Density	2.0g/cm^3
Bead Concentration	12.5mg/mL in 20% ethanol
Binding Capacity	≥40µg of 6X-His-tagged GFP/mg of beads; ≥500µg of 6X-His-tagged GFP/mL of beads



Ordering Information

Product #	Description	Pkg. Size
88831	HisPur Ni-NTA Magnetic Beads	2mL
88832	HisPur Ni-NTA Magnetic Beads	10mL

isolate **phosphopeptides** for mass spec analysis

Magnetic Phosphopeptide Enrichment

The Thermo Scientific™ Pierce™ Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit isolates phosphopeptides from complex biological samples using titanium dioxide-coated magnetic beads. The TiO_2 ligand selectively binds peptides containing phosphorylated serine (Ser), tyrosine (Tyr) or threonine (Thr), enabling phosphopeptide enrichment from protease-digested samples.

The isolated phosphopeptides are compatible for analysis downstream by mass spectrometry (MS).

Highlights

- **Complete MS-compatible kits –** include ready-to-use binding, wash and elution buffers that are optimized for phosphopeptide enrichment and downstream analysis by MALDI- and ESI-based MS
- **Optimized for high-throughput screening (HTS)** – validated procedure for processing from 1 to 96 samples at a time; complete entire assay in about 15 minutes using a KingFisher Flex Instrument
- **Stable affinity ligand** – titanium dioxide is specially coated as a film onto the magnetic particles
- **Selective** – affinity system is selective for phosphorylated Ser, Tyr and Thr; exhibits minimal nonspecific binding to acidic residues
- **Sensitive** – affinity provides more than 1000 times greater sensitivity than traditional IMAC technologies; enables enrichment and MS measurement of less than 100fmol of phosphoprotein

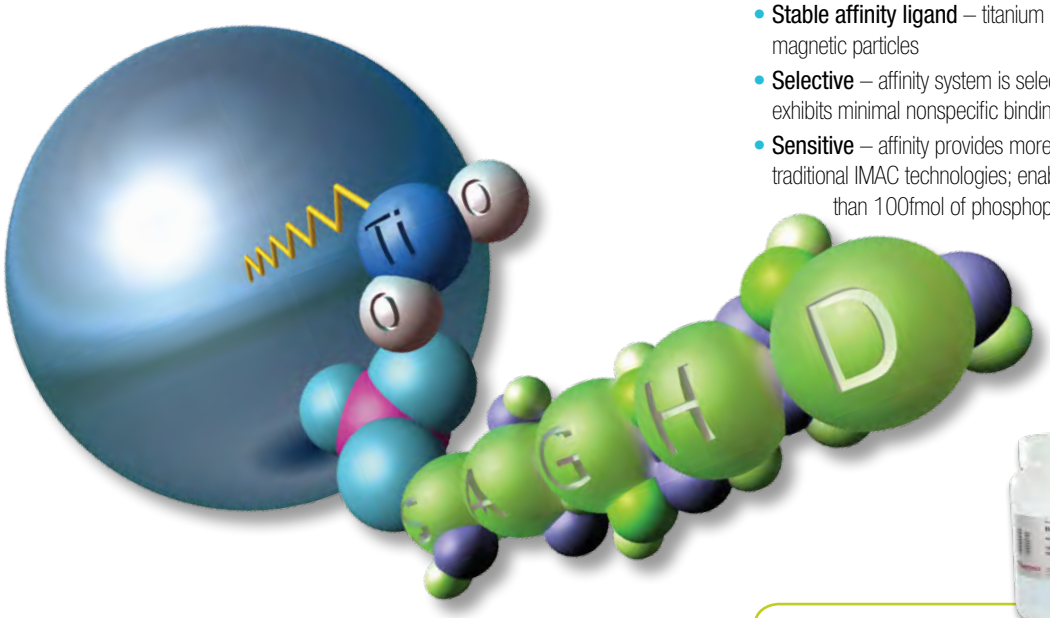


Table 9. Phosphopeptide enrichment improves MS identification of phosphoproteins. Two milligrams of a tryptic digest prepared from peripheral blood mononuclear cells (lymphocytes) with and without phosphopeptide enrichment were analyzed by MS. Enrichment was performed with the Pierce Titanium Dioxide Phosphopeptide Enrichment Kit using the KingFisher 96 Instrument. Samples were analyzed on a Thermo Scientific™ LTQ Orbitrap™ Mass Spectrometer.

	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

Ordering Information

Product #	Description	Pkg. Size
88811	**Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit** *Sufficient for: Purifying 96 x 100µg peptide samples* Contains: TiO_2 Magnetic Beads (20X), 1mL Binding Buffer, 100mL Washing Buffer, 25mL Elution Buffer, 3mL Thermo-Fast 96 Robotic PCR Plates, 0.2mL wells, 2 plates	96-rxn kit
88812	**Pierce Magnetic Titanium Dioxide Phosphopeptide Enrichment Kit, Trial Size** *Sufficient for: Purifying 24 x 100µg peptide samples* Contains: TiO_2 Magnetic Beads (20X), 0.25mL Binding Buffer, 100mL Washing Buffer, 25mL Elution Buffer, 3mL Thermo-Fast 96 Robotic PCR Plates, 0.2mL wells, 2 plates	24-rxn kit

high-throughput
quantitative proteomics

Magnetic Bead Processors and Mass Spectrometers

Our revolutionary proprietary magnetic separation technology lets you process virtually any sample from any source for the ultimate in isolation of nucleic acids, proteins and cells. With four platforms to choose from, the Thermo Scientific™ KingFisher Systems provide the performance, flexibility and speed for your budget, application and throughput requirements.

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With high-throughput or processing volume of up to 5mL, the KingFisher Flex System offers truly versatile purification of nucleic acids and proteins. Process volumes from 20µL to 5000µL, depending on the magnet head, with 96- or 24-well format. Use predefined protocols or customize your own for special applications. The new KingFisher Flex System replaces the KingFisher 96 Instrument.

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New to the KingFisher family, the KingFisher Duo System delivers advanced functionality in a compact, mid-throughput capacity instrument for isolation applications. Its small footprint and big functionality, including traceability and data management, make it a perfect fit for research and routine laboratories. Two protocols can run sequentially without interruption, raising throughput up to 24 samples per load. The KingFisher Duo System also includes large volume processing of up to 5mL.

Instrument	Flex		Duo		mL	KF
Samples/run	96	24	12(24)	6	15	24
Working volume (µL)	20-1000	200-5000	30-1000	200-5000	50-1000	20-200

For more information on Thermo Scientific KingFisher Systems, visit **thermoscientific.com/kingfisher** or consult your local sales representative.

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The Thermo Scientific KingFisher mL System enables automated, low-throughput sample preparation into your laboratory workflow. Processes volumes from 50µL to 1000µL carried out using tube strips.

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your helping hands for **protein research**

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The Cell Lysis Technical Handbook describes the latest Thermo Scientific Cell Lysis products. Included are novel lysis products for neuronal cells and synaptosomes, subcellular protein fractionation kits from tissues, new protease and/or phosphatase inhibitor tablets, and universal nuclease to reduce sample viscosity.

Mass Spec Sample Preparation Handbook

This handbook provides background, helpful hints and troubleshooting advice for cell lysis, sample preparation, detection, mass spectrometry sample preparation and downstream applications. The handbook features new products for protein concentration, purification and enrichment, plus the latest labeling techniques, including Thermo Scientific™ SILAC, TMT™, cysTMT™ and HeavyPeptide™ Reagents. The book also includes a section on Thermo Scientific Mass Spectrometry Instrumentation and Software. Everything you need to extract, digest, enrich, clean up and quantify proteins and peptides in one volume.

Western Blotting Handbook and Troubleshooting Guide

The Western Blotting Handbook and Troubleshooting Guide (version 3) details each step of the Western blotting process with technical information and products for transfer, blocking, washing, antibodies, substrates, film and stripping buffer. You will want to keep this booklet close at hand because it also includes protocols, references and a troubleshooting guide.

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