INSTRUCTIONS

Pierce™ Streptavidin Magnetic Beads

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88816 88817

Number	Description
88816	**Pierce Streptavidin Magnetic Beads**, 1mL, supplied at 10mg/mL in water containing 0.05% NaN_3
88817	**Pierce Streptavidin Magnetic Beads**, 5mL, supplied at 10mg/mL in water containing 0.05% NaN_3

Storage: Upon receipt store at 4°C. Product is shipped on an ice pack.

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Introduction

The Thermo Scientific™ Pierce™ Streptavidin Magnetic Beads provide a fast and convenient method for manual or automated immunoprecipitation, protein interaction studies, DNA-protein pulldowns, and purification of biotin-labeled proteins and nucleic acids. Biotinylated molecules are simply added to the streptavidin-coated magnetic beads for binding. A magnetic stand is used for manually removing the beads from the solution. For automated processing, the Thermo Scientific™ KingFisher™ Flex Instrument is used. This instrument is especially useful for large-scale screening of multiple samples.

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 covalently coupled to the surface of the magnetic beads. For each streptavidin molecule on the bead, there are ~3 biotin-binding sites available. Unlike avidin, streptavidin has no carbohydrate groups, resulting in low nonspecific binding. Furthermore, the magnetic beads exhibit low nonspecific binding in the presence of complex biological samples such as cell lysates. The affinity between streptavidin and biotin is high, requiring harsh conditions for disruption. It is therefore possible to elute binding partners in an interaction complex without co-eluting the biotinylated component.

Table 1. Characteristics of Thermo Scientific Pierce Streptavidin Magnetic Beads.*

Composition:	Streptavidin monolayer covalently coupled to magnetic bead surface
Magnetization:	Superparamagnetic (no magnetic memory)
Mean Diameter:	1µm (nominal)
Density:	$2 g/cm^3$
Bead Concentration:	10mg/mL
Binding Capacity:	~55µg biotinylated rabbit IgG/mg of beads; ~3500pmol biotinylated fluorescein/mg of beads

*Pierce Streptavidin Magnetic Beads are not supplied in RNase-free solutions.

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Important Product Information

- Do not freeze or dry the Pierce Streptavidin Magnetic Beads. Freezing or drying will cause the beads to aggregate and lose binding activity.
- After labeling proteins or nucleic acids with biotin, remove unincorporated biotin with a desalting column (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns, Product No. 89882-94). Free biotin will reduce binding capacity.
- To minimize protein degradation, include protease inhibitors (e.g., Thermo Scientific Halt Protease Inhibitor Single-Use Cocktail, EDTA-free, Product No. 78425) in the preparation of cell lysate.
- A low-pH elution may be used for single-use applications. To minimize streptavidin leaching, do not exceed 10 minutes for the elution step in either manual or automated protocols.
- Boiling the magnetic beads in SDS-PAGE reducing sample buffer is acceptable for single-use applications. Boiling will cause bead aggregation and loss of binding activity.
- The Pierce Streptavidin Magnetic Beads are compatible with mass spectrometry because of their low nonspecific binding.

Procedure for Manual Immunoprecipitation

A. Additional Materials Required

- 1.5mL microcentrifuge tubes
- Binding/Wash Buffer: Tris-buffered saline (TBS, Product No. 28379) containing 0.1% Tween™-20 Detergent
- Elution Buffer: IgG Elution Buffer, pH 2.0 (Product No. 21028) or 0.1M glycine, pH 2.0
- Alternate elution buffer: SDS-PAGE reducing sample buffer
- Biotinylated antibody
- Antigen sample
- Cell lysis buffer (used to prepare antigen sample)
- Magnetic stand (e.g., Thermo Scientific™ DynaMag™-2 Magnet, Product No. 12321D)

B. Pre-washing Pierce Streptavidin Magnetic Beads

Note: To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.

1. Add 50µL (0.5mg) of Pierce Streptavidin Magnetic Beads into a 1.5mL microcentrifuge tube.
2. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
3. Add 1mL of Binding/Wash Buffer to the tube. Invert the tube several times or vortex gently to mix. Collect the beads with a magnetic stand, then remove and discard the supernatant.

   Note: Do not allow beads to dry. If necessary, store beads in Binding/Wash buffer before proceeding with the purification protocol.

C. Immunoprecipitation

Note: This protocol is a general guideline for immunoprecipitation and will require optimization for each application.

1. Combine the antigen sample with 10µg of biotinylated antibody. Incubate 1-2 hours at room temperature or overnight at 4°C with mixing.

   Note: Dilute each sample to a minimum volume of 300µL with cell lysis buffer or Binding/Wash Buffer.
2. Add the antigen sample/biotinylated antibody mixture to a 1.5mL microcentrifuge tube containing pre-washed magnetic beads (see Section B above) and incubate at room temperature for 1 hour with mixing.
3. Collect the beads with a magnetic stand and remove and save the supernatant for analysis.

4. Add 300µL of Binding/Wash Buffer to the tube and gently mix. Collect the beads and then discard the supernatant. Repeat this wash twice.
5. **Elution Buffer Recovery of Antigen:** Add 100µL of Elution Buffer to the tube. Incubate the tube at room temperature with mixing for 5 minutes. Magnetically separate the beads and save the supernatant containing target antigen.

   Note: If a low pH elution buffer is selected for elution, streptavidin leaching might occur. Low pH elution buffers are effective for most antibody-antigen interactions; however, to ensure efficient release of target antigen from the antibody, pre-rinse the beads with 300µL of 0.1% Tween-20 Detergent in water (no buffering capacity) before adding Low pH Elution Buffer.

   Alternate Elution: SDS-PAGE Reducing Sample Buffer Recovery of Antigen: Add 100 µl of SDS-PAGE reducing sample buffer to the tube and heat the samples at 96-100˚C in a heating block for 5 minutes. Magnetically separate the beads and save the supernatant containing target antigen.

   Note: If SDS-PAGE buffer is selected for elution, the eluate will contain streptavidin monomers and dimers and biotinylated antibody along with target antigen.

   Note: Use the Thermo Scientific Clean-Blot IP Detection Reagent (Product No. 21230 or 21233) to prevent detection of the immunoprecipitation antibody in Western blots.

Procedure for Automated Immunoprecipitation

A. Additional Materials Required

- KingFisher Flex with 96 Deep Well Head (Product No. 5400630) Instrument
- Thermo Scientific Microtiter Deep Well 96 Plate, V-bottom, polypropylene (Product No. 95040450)
- KingFisher Flex 96 Tip Comb for Deep Well Magnets (Product No. 97002534)
- 1.5mL microcentrifuge tubes
- Binding/Wash Buffer: Tris-buffered saline (TBS, Product No. 28379) containing 0.1% Tween-20 Detergent
- Elution Buffer: IgG Elution Buffer, pH 2.0 (Product No. 21028) or 0.1M glycine, pH 2.0
- Alternative Elution Buffer: SDS-PAGE reducing sample buffer
- Antigen sample
- Biotinylated antibody

B. Preparation of the KingFisher Instrument and Plate Set-up

Note: The following protocol is for general use with the KingFisher Flex Instrument. Modify the protocol as needed using the Thermo Scientific™ BindIt™ Software provided with the instrument.

1. Combine antigen sample with 10µg of biotinylated antibody per sample. Incubate 1-2 hours at room temperature or overnight at 4˚C with mixing.
2. Download the “SA immunoprecipitation low pH elution” or “SA immunoprecipitation heated elution” protocol from the web site (*thermoscientific.com/bindit-protocols*) into the BindIt Software on an external computer.
3. Transfer the protocol to the KingFisher Flex Instrument from an external computer. See the BindIt Software User Manual for detailed instructions on importing protocols.
4. Set up the plates according to Table 2.

Table 2. Pipetting instructions for the immunoprecipitation protocol.

Plate #	Plate Name	Plate Type	Content	Volume
1	Beads	Microtiter Deep Well 96 Plate	Streptavidin Beads Binding/Wash Buffer	50µL 150µL
2	Bead Wash	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	1000µL
3	Antigen Sample	Microtiter Deep Well 96 Plate	Antibody/Antigen Sample	300µL
4	Wash 1	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	300µL
5	Wash 2	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	300µL
6	Wash 3	Microtiter Deep Well 96 Plate	Ultrapure water	300µL
7	Low-pH Elution Heated Elution	Microtiter Deep Well 96 Plate	Elution Buffer SDS-PAGE Reducing Sample Buffer	100µL
8	Tip Plate	Microtiter Deep Well 96 Plate	KingFisher Flex 96 Tip Comb for Deep Well Magnets	–

Notes:

- If using less than 96 wells, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- To ensure bead homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or rotating platform before adding the beads to Plate 1.
- Combine the Tip Comb with a Deep Well 96 Plate. See the instrument user manual for detailed instructions.
- The beads can be eluted into 100µL of 0.1M glycine, pH 2.0 or 100µL of SDS-PAGE reducing sample buffer. If using the SDS-PAGE reducing sample buffer, install the KingFisher Flex Heating Block (see manual for proper installation).
- If SDS-PAGE buffer is selected for elution, the eluate will contain streptavidin monomers and dimers and biotinylated antibody combined with target antigen.
- If a low-pH elution buffer is selected for elution, streptavidin might leach from the beads. Low-pH elution buffers are effective for most antibody-antigen interactions.

C. Executing the SA Automated Immunoprecipitation Protocol

1. Select the protocol using the arrow keys in the instrument keypad and press *Start*. See the KingFisher Flex User Manual for detailed information.
2. Slide open the door of the instrument's protective cover.
3. Load the plates into the instrument according to the protocol request, placing each plate in the same orientation. Confirm each action by pressing *Start*.
4. After the samples are processed, remove the plates as instructed by the instrument's display. Press *Start* after removing each plate.
5. Press *Stop* after all plates are removed.

Troubleshooting

Problem	Possible Cause	Solution
Low protein recovery	Proteolysis of sample	Add protease inhibitors
	Not enough magnetic beads used for capture	Increase the amount of magnetic beads used for capture
	Insufficient amount of target protein in the sample	Increase amount of antigen sample
Protein does not elute	Elution conditions were too mild	Increase incubation time with elution buffer or use more stringent elution buffer
Multiple, nonspecific bands appear in eluted sample	Nonspecific protein binding to the magnetic beads	Add 50-200mM NaCl to the Binding/Wash and Elution Buffers
Recovered protein is inactive	Elution conditions were too stringent	Use a milder elution buffer
Magnetic beads aggregate	Magnetic beads were frozen or centrifuged	Handle the beads as directed in the instructions
	The buffer was incompatible with magnetic beads	

Frequently Asked Questions for the KingFisher Instrument

Question	Answer
Which plates are compatible with KingFisher Flex Instrument?	The KingFisher Flex Instrument is compatible with the KingFisher 24 Deep Well plates, Microtiter Deep Well 96 Plates, KingFisher 96 Plates and 96 PCR Plates. The KingFisher 96 Instrument is compatible with the Microtiter 96 Deep Well Plates, KingFisher 96 Plates and 96 PCR plates.
Is it possible to concentrate samples during the run?	Both deep well plates and KingFisher 96 Plates can be used during the same run. Therefore, it is possible to start the processing by using larger volumes (in a deep well plate) and eluting the purified sample to a smaller volume (in a KingFisher 96 Plate).
Is it possible to heat the samples during the run?	The heating block is located inside the instrument and can be used automatically during the sample process. All plates compatible with the KingFisher Flex Instrument can be heated using specially designed, interchangeable heating blocks.
Why do the beads stick to the plastic tips and wells or the eluted proteins sticks to the wells?	Proteins conjugated to beads and eluted proteins can nonspecifically bind to plastics. Adding detergent in the Binding/Wash Buffer prevents the protein conjugated to the beads from sticking (e.g., 0.05%-0.1% Tween-20 Detergent). Also include a small amount of detergent in the Elution Buffer (e.g., 0.05% Tween-20 Detergent) or silanize the elution plate.
Are the reagent volumes in each well critical?	For best results, keep the specified volumes within defined limits to avoid spillover.

Additional Information Available on Our Website

- Frequently Asked Questions
- Tech Tip #43: Protein stability and storage
- Visit *thermoscientific.com/kingfisher* for information on the KingFisher Products

Related Products

88802-3	**Pierce Protein A/G Magnetic Beads**
88845-6	**Pierce Protein A Magnetic Beads**
88847-8	**Pierce Protein G Magnetic Beads**
88849-50	**Pierce Protein L Magnetic Beads**
88826-7	**Pierce NHS-Activated Magnetic Beads**
78601	**Pierce Glutathione Magnetic Agarose Beads**
A32993	**High-Select™ TiO_2 Phosphopeptide Enrichment Kit**
A32992	**High-Select™ Fe-NTA Phosphopeptide Enrichment Kit**
24615	**Imperial™ Protein Stain**
34075	**SuperSignal™ West Dura Extended Duration Substrate**
XP04202BOX	**Novex™ Tris-glycine protein gels** (see thermofisher.com/proteingels for a complete listing)
NW04122BOX	**Bolt™ Bis-Tris Plus protein gels** (see thermofisher.com/proteingels for a complete listing)
21955	**EZ-Link™ Micro NHS-PEG_4-Biotinylation Kit**

Cited Reference

1. Chaiet, I. and Wolf, F.J. (1964). The properties of streptavidin, a biotin-binding protein produced by Streptomycetes. *Arch Biochem Biophys* **106**:1-5.

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