

Pierce Magnetic ChIP Kit

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26157

Number	Description
26157	Pierce Magnetic ChIP Kit , contains sufficient reagents to perform 30 ChIP reactions

Kit Contents:**4°C Components for Magnetic ChIP Kit (26157X)**

Glycine Solution (10X), 15mL; store at room temperature

PBS (20X), 15mL; store at 4°C

Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X), 1mL; store at 4°C

Membrane Extraction Buffer, 10mL; store at 4°C

MNase Digestion Buffer, 10mL; store at 4°C

DTT (7.7mg), Lyophilized, 2 vials; store at room temperature or 4°C

MNase Stop Solution, 1mL; store at 4°C

ChIP-Grade Protein A/G Magnetic Beads, 0.7mL; store at 4°C

IP Dilution/Wash Buffer (5X), 40mL, store at 4°C

Sodium Chloride (5M), 6mL; store at 4°C

IP Elution Buffer (2X), 4.5mL; store at 4°C

Microcentrifuge Tubes, 1.5mL, 75 tubes; store at room temperature or 4°C

DNA Clean-Up Column and Reagents, 40 purifications; store at room temperature or 4°C

DNA Clean-Up Column, 40 columns

DNA Column Binding Solution, 30mL

DNA Column Wash Solution, 6mL

pH Indicator, 0.8mL

DNA Column Elution Solution, 5mL

-20°C Components for Magnetic ChIP Kit (1862739) (yellow bag)

Micrococcal Nuclease (ChIP Grade) (10U/μL), 50μL; store at -20°C

Anti-RNA Polymerase II Antibody, 25μL; store at -20°C

Normal Rabbit IgG (1mg/mL), 10μL; store at -20°C

Proteinase K (20mg/mL), 0.25mL; store at -20°C

ChIP Positive Control Primers (GAPDH promoter; human specific), 100μL; store at -20°C

Storage: Upon receipt store contents of yellow bag at -20°C. Store all other components as indicated. The kit is shipped at ambient temperature.

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Introduction

The Thermo Scientific™ Pierce™ Magnetic ChIP Kit provides a simple, fast and reproducible method to perform chromatin immunoprecipitation (ChIP) assays. ChIP assays identify links between the genome and the proteome by monitoring transcription regulation through histone modification¹ (epigenetics) or transcription factor-DNA binding interactions. The strength of the ChIP assay technique is its ability to capture a snapshot of specific protein-DNA interactions as they occur in living cells and then quantitate the interactions using standard or quantitative PCR.^{2,3}

The kit contains all reagents to perform a successful ChIP assay. The specially titrated and tested micrococcal nuclease digests the DNA, eliminating variable results caused by the traditional method of shearing DNA by sonication. The advantages of enzymatic digestion include reproducibility of digestion, control of the reaction and easy titration of the enzyme for each specific cell type. The specially blocked ChIP-grade Thermo Scientific Pierce Protein A/G Magnetic Beads provide high-binding capacity, low background and compatibility with ChIP sequencing. The immunoprecipitation (IP) and elution portions are compatible with automated platforms such as the Thermo Scientific™ KingFisher™ Instruments. Using the optimized reagents and protocol, the Pierce Magnetic ChIP Kit is streamlined to achieve complete crosslink reversal, protein digestion and DNA purification with minimal time and sample handling.

Additional Materials Required

- Mammalian cell culture reagents and equipment
- Chemical fume hood
- ChIP-qualified primary antibody against the DNA-binding protein of interest
- Cell scrapers
- Thermomixer or heat block
- 50mL and 15mL conical tubes
- 16% formaldehyde (Product No. 28906)
- Micro-tip probe sonicator (e.g., Misonix™ Sonicator™ 3000 Instrument)
- Magnetic stand for microcentrifuge tubes
- Nuclease-free water
- Ethanol (95-100%)
- Standard or quantitative PCR reagents and equipment

- Oligonucleotide primers specific to the gene promoter of interest
- If using KingFisher instruments for automation:
 - Thermo Scientific™ KingFisher™ Flex System with 96 deep well head (Product No. 5400630)
 - Thermo Scientific™ Microtiter Deep Well 96 Plate, V-bottom, polypropylene (Product No. 95040450)
 - Thermo Scientific™ KingFisher™ Flex 96 Tip Comb for Deep Well Magnets (Product No. 97002534)

Important Product Information

- **Read the entire protocol before beginning the assay.**
- The recommended starting range for cells per ChIP reaction is 2×10^6 to 4×10^6 . The kit will accommodate 30 ChIP reactions at 4×10^6 cells each (see Table 1 for appropriate reagent volumes).
- When designing a ChIP experiment, consider the number of immunoprecipitations desired from the chromatin sample and the number of cell culture conditions (e.g., drug-treated vs. non-treated). For accurate comparison, immunoprecipitate the same target proteins and controls from each cell culture condition.
- For a positive control, perform an immunoprecipitation on chromatin from the control cell culture condition using the included Anti-RNA Polymerase II Antibody, then amplify the resulting DNA with the provided GAPDH primer pair. Performing a ChIP using the Normal Rabbit IgG is an effective negative control.
- If performing multiple ChIPs from a single cell culture condition, crosslinked chromatin may be prepared in bulk. Scale the reagent amounts according to the number of ChIPs desired and sub-divide the chromatin sample after sonication. The total input control obtained from the cell culture condition is common to this set of ChIPs.

Procedure Summary

1. Crosslink and harvest cells.
2. Lyse membrane and cytosol.
3. Digest with MNase.
4. Lyse nuclei by sonication to obtain chromatin.
5. Incubate chromatin with antibody.
6. Add Protein A/G magnetic beads.
7. Wash.
8. Elute.
9. Crosslink reversal and treatment with Proteinase K.
10. DNA clean-up.
11. qPCR and/or sequencing.

Material Preparation

Note: Prepare the reagents listed below once and use for all 30 ChIP reactions.

Glycine Solution (10X)	If precipitate formed, warm solution to 37°C in a water bath for 30 minutes and vortex before use.
PBS	If precipitate formed, warm solution to 37°C in a water bath for 30 minutes and vortex before use. Dilute PBS (20X) to 1X with nuclease-free water.
1M DTT	Add 50µL of nuclease-free water to one tube of DTT. Store this solution at -20°C for up to 6 months.
DNA Column Binding Buffer	Add 120µL of pH Indicator Solution to the vial of 30mL DNA Column Binding Solution and mix well. Store at room temperature.

DNA Column Wash Buffer	Add 24mL of ethanol (95-100%) into the provided vial of 6mL DNA Column Wash Solution and mix well. Store at room temperature.
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Note: The amounts listed below are for preparing one ChIP. If you are preparing chromatin in bulk or performing multiple ChIPs, multiply the reagent amounts by the number of ChIPs being performed (Table 1).

Membrane Extraction Buffer	Add 2µL of Halt Cocktail to 200µL of Membrane Extraction Buffer in a microcentrifuge tube and place on ice.
MNase Digestion Buffer Working Solution	Add 0.21µL of 1M DTT to 210µL of MNase Digestion Buffer and place tube at room temperature.
1X IP Dilution Buffer	Add 100µL of IP Dilution/Wash Buffer (5X) and 5µL of Thermo Scientific™ Halt™ Cocktail to 395µL of nuclease-free water. Store at 4°C.
IP Wash Buffer 1	Mix 0.6mL of IP Dilution Buffer/Wash Buffer (5X) with 2.4mL of nuclease-free water. Store at 4°C.
IP Wash Buffer 2	Mix 200µL of IP Dilution/Wash Buffer (5X) and 70µL of sodium chloride (5M) with 730µL of nuclease-free water. Store at 4°C.
1X IP Elution Buffer	Warm the IP Elution Buffer (2X) in a 37°C water bath until fully dissolved. For each IP and total input control, prepare 150µL of 1X IP Elution Buffer by combining 75µL of IP Elution Buffer (2X) with 75µL of nuclease-free water. Store at room temperature.

ChIP Procedure

A. Crosslinking and Cell Pellet Isolation

Note: This procedure is for one ChIP using 4×10^6 cells. Multiply reagent amounts by the number of ChIPs being performed.

Table 1. Reagent volumes to use based on the number of ChIPs.

<u>Example number of HeLa cells</u>	<u>$< 2 \times 10^6$</u>	<u>4×10^6</u>
Number of ChIPs	1	1
1X PBS	2.1mL	4.2mL
Lysis Buffer 1	0.1mL	0.2mL
MNase Digestion Buffer Working Solution	0.1mL	0.2mL
1X IP Dilution Buffer	0.5mL	
IP Wash Buffer 1	3mL	
IP Wash Buffer 2	1mL	
1X IP Elution Buffer	0.15mL	

1. Culture adherent mammalian cells and treat as desired.

Optional: If you are unfamiliar with the cell type being used, culture an extra dish of cells for determining cell number. Before crosslinking, trypsinize and determine the cell number from the extra dish of cells.

2. To each dish containing cell culture media, add sufficient quantity of 16% formaldehyde to obtain a final concentration of 1% formaldehyde.

Caution: Formaldehyde is a skin irritant and inhalation hazard with toxic fumes. Use proper personal protective, laboratory safety and disposal equipment.

3. Mix well by gently swirling the dish. Incubate at room temperature for 10 minutes in a chemical fume hood.
4. To each dish containing cell culture media and formaldehyde, add Glycine Solution (10X) to a final concentration of 1X. Mix well by gently swirling the dish. Incubate at room temperature for 5 minutes in the chemical fume hood.
5. Aspirate formaldehyde/glycine-containing media in a fume hood. Properly dispose of formaldehyde-containing waste.
6. Wash the cells twice with one media volume of ice-cold PBS, removing each wash by aspiration.

7. Add 10 μ L of the Halt Cocktail to 1mL of ice-cold PBS. Add the solution to the cells and detach cells by scraping. Transfer the cell suspension to a 1.5mL microcentrifuge tube using a pipette.
8. Centrifuge tubes at 3000 \times g for 5 minutes.
9. Remove the PBS. Store the cell pellet(s) at -80°C or proceed directly to Section B: Lysis and MNase Digestion.

B. Lysis and MNase Digestion

For best results, empirically determine the optimal crosslinking time and MNase digestion conditions for each cell type (Appendix A). The typical range of diluted MNase per 4×10^6 cells is 1.5-2.5 μ L. The amount of MNase needed will also depend on the desired fragment size. The following cell lines have been tested to show optimal digestion (i.e., bands equally distributed from 150bp to ~1000bp) using 2 μ L of diluted MNase per 4×10^6 cells at 37°C for 15 minutes: HeLa, A431, HepG2, LNCap and 3T3. The amount of MNase used scales accordingly with cell number (for example, if 2×10^6 HeLa cells are used, only 1 μ L of diluted MNase is needed.)

Note: The volumes listed in this protocol are for 4×10^6 cells. Refer to Table 1 for other cell numbers.

1. Use the crosslinked cells prepared above. If frozen, thaw cells on ice.
2. Add 200 μ L of Membrane Extraction Buffer containing protease/phosphatase inhibitors to the cell pellet and pipette up and down to break up the pellet. Vortex the tube for 15 seconds and incubate on ice for 10 minutes.
3. Centrifuge at 9000 \times g for 3 minutes and remove the supernatant.
4. Resuspend nuclei in 200 μ L of MNase Digestion Buffer Working Solution.
5. Dilute the MNase (ChIP grade) (10U/ μ L) by adding 0.5 μ L to 4.5 μ L MNase Digestion Buffer Working Solution. Pipette up and down to mix well.

Note: Do not store diluted MNase. Discard after use.

6. Add 2 μ L of diluted MNase, vortex the tube and incubate in a 37°C water bath for 15 minutes, mixing by inversion every 5 minutes.
7. Add 20 μ L of MNase Stop Solution to stop the reaction, vortex briefly and incubate on ice for 5 minutes.
8. Centrifuge at 9000 \times g for 5 minutes to recover the nuclei. Remove the supernatant.
9. Resuspend nuclei in 100 μ L of 1X IP Dilution Buffer containing protease/phosphatase inhibitors.
10. Sonicate on ice with several pulses to break nuclear membrane. Incubate for 20 seconds on ice between pulses.

Note: Optimal conditions for complete lysis can be monitored by observing under a light microscope. For 4×10^6 HeLa, A431, HepG2, LNCap and 3T3 cells, use three 20-second pulses at 3 watts power (using Sonicator 3000 Instrument). Do not sonicate more than 500 μ L at one time.

11. Centrifuge at 9000 \times g for 5 minutes and transfer the supernatant, containing the digested chromatin, to a new 1.5mL tube. Proceed to the immunoprecipitation or store samples at -80°C.

C. Immunoprecipitation

Note: Ensure that all buffers are cold and stored on ice for use.

1. Transfer 10 μ L of the supernatant containing the digested chromatin to a 1.5mL tube and store at -20°C. This is the 10% total input sample from one ChIP.

Note: If you are preparing chromatin in bulk, this sample can be used as a common input control for all IPs from the bulk preparation.

2. Transfer the remaining 90 μ L of supernatant to 410 μ L of 1X IP Dilution Buffer.
3. Add primary antibody. For optimal results when using $2-4 \times 10^6$ HeLa cells/IP, use the following amounts of antibody:

Positive control IP (included): add 10 μ L of Anti-RNA Polymerase II Antibody

Negative control IP (included): add 1-2 μ L of Normal Rabbit IgG

Target-specific IP(s): Typical concentration is 1-10 μ g antibody for each IP; however, titration of antibody concentration to obtain the best signal-to-noise ratio is required.

4. Incubate IP reactions for 2 hours to overnight at 4°C with mixing.
Note: For low-abundant proteins, an overnight incubation greatly increases signal.
5. Vortex the tube of ChIP Grade Protein A/G Magnetic Beads to obtain a uniform suspension. Add 20µL of the magnetic beads to each IP and incubate for 2 hours at 4°C with mixing.
6. After incubation, collect the beads with a magnetic stand and carefully remove and discard the supernatant.
7. Add 1mL of IP Wash Buffer 1, vortex briefly and incubate for 5 minutes with mixing.
8. Collect the beads with a magnetic stand and carefully remove and discard the supernatant.
9. Perform Steps 7 and 8 a total of three times with IP Wash Buffer 1.
10. Perform Steps 7 and 8 once with IP Wash Buffer 2.

D. IP Elution

1. Add 150µL of 1X IP Elution Buffer to the washed beads, cap and incubate at 65°C for 30 minutes with vigorous shaking. If a thermomixer is unavailable, incubate the column in a heat block set at 65°C for 40 minutes. Resuspend the beads by vortexing the tube every 10 minutes.
2. During the elution step, prepare a 1.5mL microcentrifuge tube for each IP containing 6µL of 5M NaCl and 2µL of 20mg/mL Proteinase K.
3. Thaw 10% total input sample(s) and add 150µL of 1X IP Elution Buffer, 6µL of 5M NaCl and 2µL of 20mg/mL Proteinase K. Place tubes at room temperature until the next step.
4. Following the 65°C incubation, remove the tube from the heat block and collect the beads with a magnetic stand.
5. Remove the supernatant (containing the eluted protein-chromatin complex) and dispense into prepared tubes with the NaCl and Proteinase K. Cap the 1.5mL centrifuge tubes, vortex all IP and total-input samples, and place in a 65°C heat block for 1.5 hours.

E. DNA Recovery

1. To each eluted IP and total input sample, add 750µL of DNA Binding Buffer.
2. Pipette 500µL of each sample into a DNA Clean-Up Column inserted into a 2mL collection tube. Centrifuge the columns at 10,000 × g for 1 minute and discard the flow-through.
3. Pipette the remaining sample into the same DNA Clean-Up Column. Centrifuge the columns at 10,000 × g for 1 minute and discard the flow-through.
4. Place the column back into the collection tube and add 750µL of DNA Column Wash Buffer. Centrifuge the columns at 10,000 × g for 1 minute and discard the flow-through.
5. Place the column back in the empty collection tube and centrifuge the columns at 10,000 × g for 2 minutes.
6. Place the column in a new 1.5mL centrifuge tube and pipette 50µL of DNA Column Elution Solution directly into the center of each column.
7. Centrifuge the column at 10,000 × g for 1 minute and discard the column. The resulting solution is the purified DNA. Proceed to PCR or qPCR detection (Appendix B).

Troubleshooting

Problem	Possible Cause	Solution
Chromatin fragments are too large (> 1000 base pairs)	Crosslinking time was too long	Shorten crosslinking time
	Cell to MNase ratio was too high	Increase amount of MNase or decrease cell number (refer to the MNase digestion optimization protocol in Appendix A)
Chromatin fragments are too small (< 200 base pairs)	Cell to MNase ratio was too low	Decrease amount of MNase or increase cell number (refer to the MNase digestion optimization protocol in Appendix A)
No or low PCR signal in the total input control samples	PCR amplification conditions were not fully optimized	Optimize PCR conditions using samples known to contain the target amplicon Check primer design
	Insufficient amount of sample DNA added to the PCR reaction	Increase the amount of sample DNA added to the PCR reaction
	Nuclei not fully lysed	Monitor sonication of nuclei by microscope to ensure full lysis
No or low PCR signal in the positive control IP samples	Insufficient chromatin amount in the IP reaction	Use at least 25µg of chromatin for each IP
	Insufficient antibody incubation time	Incubate antibody overnight
	Nuclei not fully lysed	Monitor sonication of nuclei by microscope to ensure full lysis
	Low abundant target	Add more chromatin or magnetic beads (30µL)
PCR signal of the positive and negative control IP samples are equivalent	Excess chromatin or antibody added to the IP	Add less chromatin or antibody
	PCR amplification was measured outside the linear range of amplification	Decrease the number of amplification cycles used in the PCR reaction
	Insufficient amount of sample DNA added to the PCR reaction	Add more sample DNA to the PCR reaction
No or low PCR signal in the experimental IP samples	Insufficient amount of antibody added to the IP	Add more antibody to the IP
	Antibody did not function in an IP	Verify that the antibody is qualified for ChIP or IP applications and has been handled and stored properly

Appendix A: Optimization of MNase Digestion

1. Prepare crosslinked cell pellet from 10^7 cells as described in Section C.
2. Follow the lysis procedure in Section B, Steps 1-4; however, use 0.5mL of the Membrane Extraction Buffer and MNase Digestion Buffer.
3. Transfer 100µL of the resuspended nuclei into five tubes (2×10^6 nuclei/tube), labeling the tubes 0, 1, 2, 4 and 6.
4. Add 2µL of MNase (ChIP-grade) to 18µL of MNase Digestion Buffer (1:10 dilution).
5. Add 0, 1, 2, 4 and 6µL of the diluted MNase to the corresponding tubes of nuclei and incubate for 15 minutes in a 37°C water bath mixing by inversion every 5 minutes.
6. Add 20µL of MNase Stop Solution to each tube and incubate on ice for 5 minutes.
7. Centrifuge at $9000 \times g$ for 5 minutes to recover the nuclei and remove the supernatant.
8. Resuspend nuclei in 100µL of 1X IP Dilution Buffer.
9. Sonicate on ice with several pulses to break nuclear membrane. Incubate for 20 seconds on ice between pulses.

Note: Optimal conditions for complete lysis may be monitored by observing under a light microscope. For 4×10^6 HeLa, A431, HepG2, LNCap and 3T3 cells, use three 20-second pulses at 3 watts power (using Sonicator 3000 Instrument).

10. Centrifuge at $9000 \times g$ for 5 minutes.
11. Prepare five 1.5mL microcentrifuge tubes labeled 0, 2, 4 and 6, each containing 6.6 μ L of nuclease-free water, 2.4 μ L of 5M NaCl and 1 μ L of 20mg/mL Proteinase K.
12. Transfer 100 μ L of each supernatant containing digested chromatin to the corresponding tube.
13. Vortex each tube for 10 seconds and incubate the tubes at 65°C in a heat block for 1.5 hours.
14. Analyze 15-25 μ L of each sample and DNA size marker by agarose gel electrophoresis. Optimal digestion yields fragments from 200 to 1000 base pairs with a more intense ladder of bands occurring at approximately 160, 320 and 480 base pairs, which corresponds to the 1, 2 and 3 nucleosome units.

Note: The optimal amount of diluted MNase determined by the above procedure is for 2×10^6 cells. Scale the MNase according to cell number (i.e., if 1 μ L was determined to be optimal for 2×10^6 cells, use 2 μ L for 4×10^6 cells).

Appendix B: Real-Time PCR Analysis Using Positive Control Primers

- Use filter tips, gloves and nuclease-free reagents to prevent contamination.
- Using hot-start Taq polymerase and validated PCR master mix greatly reduces PCR artifacts and increases reaction efficiency.
- The included Positive Control Primers are designed to amplify a region of the human GAPDH promoter close to the transcription start site. The primers are validated for detecting Anti-RNA Polymerase II Antibody binding to the GAPDH promoter in human-derived cells and tissue (Table 2).
- When designing primers, using reputable primer design software greatly increases successful PCR detection.
- Follow the manufacturer’s recommendations when programming the thermocycler and collecting real-time data.
- Use a standard curve of serially diluted genomic DNA to evaluate PCR efficiency and linear amplification.
- Amplify each sample in triplicate to control for pipetting error.

Table 2. Recommended reaction conditions for quantitative real-time PCR (qPCR) with the GAPDH control.

Reagent	Volume/reaction (μ L)
Thermo Scientific™ 2X ABSolute qPCR SYBR™ Green Fluorescein Mix	12.5
ChIP Positive Control Primers (GAPDH Promoter)	1.0
Template DNA	5.0
Nuclease-free Water	6.5

Thermocycler Amplification Settings:

Step 1: 95°C for 15 minutes.

Step 2: 95°C for 15 seconds.

Step 3: 62°C for 1 minute (collect real-time data).

Step 4: Repeat Steps 2 and 3 for 40 cycles.

Appendix C: Automated Immunoprecipitation

Note: The following protocol is designed for general use with the KingFisher Flex or KingFisher 96 Instrument. The protocol can be modified according to customer needs using the Thermo Scientific™ BindIt™ Software provided with the instrument.

1. Download the “Magnetic ChIP” protocol from the Thermo Scientific website (<http://www.thermoscientific.com/bindit-protocols>) into the BindIt Software on an external computer.
2. Transfer the protocol to the KingFisher Flex or KingFisher 96 Instrument from an external computer. See the BindIt Software User Manual for detailed instructions on importing protocols.

- Perform protocol as listed above through Step C5 (formation of the bead-antibody-protein-DNA complex)
- Set up the plates according to Table 3.

Table 3. Pipetting instructions for the ChIP protocol using the Thermo Scientific Microtiter Deep Well 96 Plates.

Plate #	Plate Name	Content	Volume
1	IP	Bead-antibody-protein-DNA complex	500µL
2	Wash 1		
3	Wash 2	IP Wash Buffer 1	1000µL
4	Wash 3		
5	Wash 4	IP Wash Buffer 2	1000µL
6	Elution	1X Elution Buffer	150µL
7	Tip Plate	KingFisher Flex 96 Tip Comb for Deep Well Magnets	-

- Select the instrument protocol and load each plate in the same orientation. Press Start.
- After the samples are processed, remove the plates as instructed.
- Proceed to Step D2 to reverse the crosslinks and Proteinase K treatment.

Frequently Asked Questions for the KingFisher Instrument

Question	Answer
Which plates are compatible with KingFisher Flex and KingFisher 96 Instruments?	The KingFisher Flex Instrument is compatible with the KingFisher 24 Deep Well Plates, Microtiter Deep Well 96 Plates and KingFisher 96 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 using larger volumes (in a deep-well plate) and elute 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 may 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 stick to the wells?	Eluted proteins and proteins conjugated to beads can nonspecifically bind to plastics. Adding detergent to the Binding/Wash Buffer prevents the protein conjugated to the bead 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

Related Thermo Scientific Products

See www.thermoscientific.com/pierce-antibodies for a full listing of ChIP-validated antibodies.

26162	ChIP-Grade Protein A/G Magnetic Beads
26158	Pierce™ Chromatin Prep Module
26156	Pierce™ Agarose ChIP Kit
26159	ChIP-Grade Protein A/G Plus Agarose
28908	16% Formaldehyde (w/v), Methanol-free
28348	20X Phosphate Buffered Saline

26160	Proteinase K Solution (20mg/mL)
78443	Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-Free (100X)
78833	NE-PER™ Nuclear and Cytoplasmic Extraction Kit
20148	LightShift™ Chemiluminescent EMSA Kit
23225	Pierce™ BCA Protein Assay Kit
AB-0900*	Thermo-Fast™ 96-Well Semi-Skirted PCR Plate, opaque white
AB-1170*	ABsolute™ QPCR Seal

* Products can be found at www.thermoscientific.com

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