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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Zymo-Spin™ ChIP Kit

(Mechanical Shearing Protocol)

Catalog Nos. **D5209 & D5210**

Highlights

- Unique workflow features a micro-elution (≥6 µl) spin column for purification of ChIP DNA.
- High quality ChIP DNA is ideal for ChIP-qPCR, ChIP-Seq, and other molecular applications.

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

Product Contents:

Zymo-Spin™ ChIP Kit	D5209 (10 Preps.)	D5210 (25 Preps.)	Storage Temperature
Nuclei Prep Buffer	30 ml	30 ml	RT
Chromatin Shearing Buffer	30 ml	30 ml	RT
Chromatin Dilution Buffer	30 ml	30 ml	RT
ZymoMag Protein A[1]	200 µl	400 µl	4°C
Chromatin Wash Buffer I (green label)	30 ml	30 ml	RT
Chromatin Wash Buffer II (green label)	30 ml	30 ml	RT
Chromatin Wash Buffer III (green label)	30 ml	30 ml	RT
5X Chromatin Elution Buffer[2]	10 ml	10 ml	RT
5 M NaCl	1 ml	1 ml	RT
Proteinase K[3]	1 mg	1 mg	-20°C
ChIP DNA Binding Buffer	10 ml	50 ml	RT
DNA Wash Buffer[4] (yellow label)	6 ml	6 ml	RT
DNA Elution Buffer	10 ml	16 ml	RT
Zymo-Spin™ IC	10	25	RT
Collection Tubes (2 ml)	10	25	RT
Magnetic Rods	4	4	-
Instruction Manual	1	1	-

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

The ChIP buffers and ChIP DNA clean-up technologies are provided at room temperature (Component 1 of 2). Cold components are provided in a ice box (Component 2 of 2).

[1]Upon arrival, store **ZymoMag Protein A** at 4°C.

[2]**5X Chromatin Elution Buffer** contains RNase A at a concentration of 85 µg/ml.

[3]Add 50 µl **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K (D3001-2-1)** at 20 mg/ml prior to use and store at -20°C.

[4]Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **DNA Wash Buffer** concentrate prior to use.

Specifications:

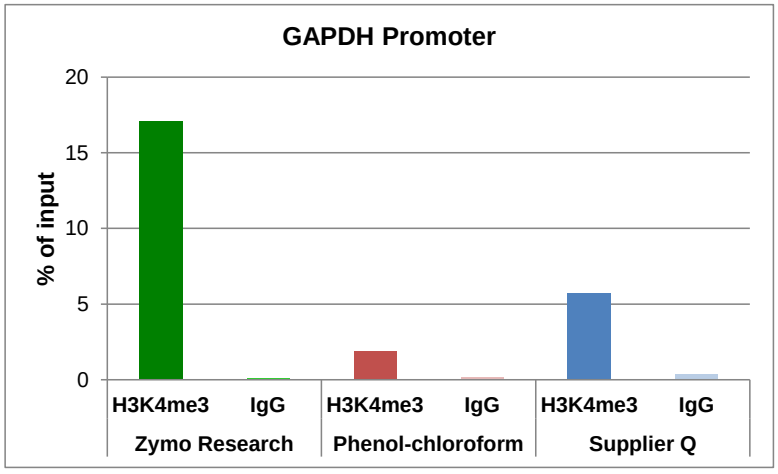
- **Compatibility:** This kit contains reagents optimized for mammalian cells.
- **User Supplied Reagents:** Formaldehyde (37% stock, w/v), Glycine (2.5 M), Phosphate Buffered Saline, Protease inhibitor cocktail, Phenylmethylsulfonyl Fluoride (0.1 M), Ethanol (95% or 100%), ChIP-grade antibody, Quest*Taq*™ qPCR Premix.
- **Required Equipment:** Sonicator, magnetic tube rack, vortex, tube rotator, microcentrifuge, agarose gel apparatus/Bioanalyzer/TapeStation, qPCR instrument.

Product Description:

The field of epigenetics has grown tremendously over the past several decades. Studies have shown that chromatin modifications, DNA methylation, and DNA hydroxymethylation play critical roles in the regulation of gene expression, gene silencing, protein-DNA interactions, and other cellular processes. Dysregulation of epigenetic modifications can contribute to developmental abnormalities, neurological disorders, and even cancer.

Chromatin immunoprecipitation (ChIP) is the prevailing method used for the study of protein-DNA interactions and the dynamics of epigenetic modifications. ChIP facilitates the identification of regions of the genome associated with a specific protein. The **Zymo-Spin™ ChIP Kit** from Zymo Research provides a streamlined ChIP procedure for investigating protein-DNA interactions that have been "fixed" in their natural state and can be used to effectively identify binding sites for transcription factors, co-factors, and other DNA regulatory proteins.

Briefly, this ChIP protocol involves covalent cross-linking of protein-DNA complexes with formaldehyde followed by cell lysis and chromatin shearing. A ChIP-grade antibody (*user supplied*) is used with Protein A magnetic beads to immunoprecipitate the protein-DNA complexes of interest. Following reverse crosslinking, RNase A and Proteinase K treatments, the DNA is eluted in a minimal volume of buffer using a unique micro-elution spin column, eliminating the need for messy precipitations. The protocol has been optimized for efficient crosslinking, shearing, and immunoprecipitation regardless of the mammalian cell type. Additionally, eluted ChIP DNA is ideal for end point PCR, quantitative PCR, ChIP-Seq library preparation, and Next-Gen sequencing-based applications.



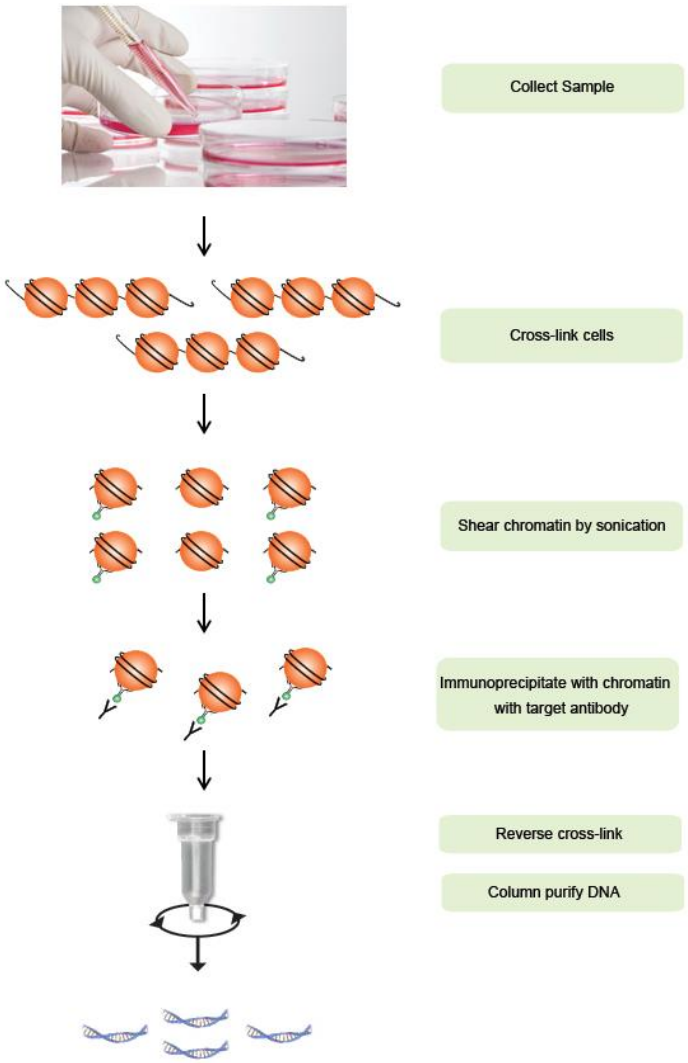
ChIP DNA Purification Method	Zymo Research	Phenol-chloroform	Supplier Q
Total Time	5 min.	>60 min.	5 min.

ChIP DNA Purification Comparison
ChIP assays were performed with HeLa cells and ChIP-grade anti-H3K4me3 and rabbit IgG. Both total and immunoprecipitated chromatin were reverse cross-linked and recovered using either the ChIP DNA Clean & Concentrator™ (included in the Zymo-Spin™ ChIP Kit), phenol-chloroform extraction, or DNA recovery kit from Supplier Q. The amount of ChIP DNA was determined using qPCR with primers specific to the GAPDH promoter. ChIP DNA enrichment is graphed as % input (i.e., the relative amount of immunoprecipitated DNA compared to input DNA following qPCR analysis).

Considerations for Experimental Design:

- **Cell number –** This protocol has been optimized for 1 x10^6 cells per 1 ml ChIP reaction. To process more cells, prepare separate ChIP reactions and then pool the eluted ChIP DNA. For smaller inputs, scale down the reaction volume proportionally (e.g., 1 x10^5 cells for a 100 µl ChIP reaction).
- **Chromatin Shearing –** This protocol has been optimized with HeLa cell chromatin mechanically sheared using a Branson Sonifier®. Before beginning the ChIP procedure, chromatin needs to be sheared to 100–600 bp in a volume of 300–500 µl per sonication. Although the conditions given in the protocol should be adequate for a variety of cell types, it is recommended that an initial evaluation of shearing efficiency be conducted prior to ChIP as detailed in **APPENDIX A**.

Outline of the Zymo-Spin™ ChIP Kit Procedure



Protocol:

SECTION 1 *and* ***SECTION 2*** *below describe formaldehyde cross-linking and chromatin shearing, respectively, with the input of 5 x10^6 cells. This cellular input will be sufficient for 5 ChIP reactions (Note: If working with more cells, scale up accordingly).*

SECTION 1: Cell Collection and Formaldehyde Cross-linking

1. Harvest cells using appropriate method (e.g., trypsin) and then wash cells twice with 1X PBS.

2. Count cells and resuspend 5 x10^6 cells in 1 ml of 1X PBS.

3. Cross-link cells by adding formaldehyde (user provided) to a final concentration of 1% (v/v) (e.g., add 27 µl of a 37% formaldehyde solution to 1 ml cell suspension). Incubate for 7 min at room temperature with gentle shaking or rotating.

4. Stop the cross-linking reaction by adding glycine (user provided) to a final concentration of 0.125 M (e.g., add 50 µl of 2.5 M Glycine to 1 ml cell suspension) and continue shaking or rotating for 5 min.

   Important! For Steps 5 to 20, all buffers, tubes, and centrifugation steps must be kept at ~4 °C.

5. Centrifuge cross-linking reaction at 3,000 *x g* for 1 min at *4 °C*.

6. Discard supernatant and resuspend cell pellet in 1 ml chilled 1X PBS containing 1 mM phenylmethylsulfonyl fluoride (PMSF)[1] and 1X protease inhibitor cocktail (PIC)[2] (e.g., add 10 µl of 0.1 M PMSF and 10 µl of 100X PIC to 1 ml chilled 1X PBS).

   Note: *Additional protease inhibitors can be added depending on the application: For histone acetylation targets add 10 mM sodium butyrate (HDAC inhibitor) and other inhibitors can be added if required.*

7. Centrifuge at 3,000 *x g* for 1 min at *4 °C* and repeat Steps 6 & 7.

8. Discard the supernatant and continue with **Section 2: Nuclei Preparation and Chromatin Shearing**.

   Optional: *Cross-linked pellets can be stored at -80 °C for future use.*

Notes:

[1]PMSF has a half-life of approximately 30 minutes in aqueous solutions and should be added just prior to use.

[2]Example Protease Inhibitor Cocktails (PICs) include: Halt Protease Inhibitor Cocktail (Thermo Scientific, Cat. No. PI78437), Protease Inhibitor Cocktail (Sigma-Aldrich, Cat. No. P2714), or similar.

Notes:

[1]This protocol is compatible with the majority of mammalian cells. However optimization of cross-linking and shearing conditions for a specific cell type may be required.

[2]An initial evaluation of shearing efficiency should be conducted prior to ChIP with the sonicator being used for the experiment, please refer to **APPENDIX A**.

SECTION 2: Nuclei Preparation and Chromatin Shearing[1]

9. Resuspend the cross-linked cell pellet in 500 µl chilled **Nuclei Prep Buffer** containing 1 mM PMSF and 1X PIC (e.g., add 5 µl of 0.1 M PMSF and 5 µl of 100X PIC to 500 µl chilled **Nuclei Prep Buffer)**.

10. Vortex briefly to ensure cell pellet is completely resuspended and incubate on ice for 5 min.

11. Centrifuge at 3,000 *x g* for 1 min at *4 °C*.

12. Discard supernatant and resuspend cell pellet in 500 µl chilled **Chromatin Shearing Buffer** containing 1mM PMSF and 1X PIC (e.g. add 5 µl of 0.1 M PMSF and 5 µl of 100X PIC to 500 µl chilled **Chromatin Shearing Buffer)**.

    Important! *Do not vortex, resuspend cell pellet by pipetting.*

    Note: *If you noticed a precipitate in Chromatin Shearing Buffer, warm the buffer **briefly** by hand until the precipitate is dissolved.*

13. Incubate on ice for 5 min.

14. Sonicate on ice for 4 cycles (30 sec “ON”, 30 sec “OFF” at 40% amplitude). Tip must be submerged (~¾ inch) in the buffer to avoid foaming[2].

15. Centrifuge at 12,000 *x g* for 5 min and then transfer the supernatant containing the sheared chromatin (~500 µl) to a pre-chilled 1.5 ml tube.

SECTION 3: Immunoprecipitation with ZymoMag Protein A

This protocol has been optimized for 100 µl inputs of sheared chromatin (~1 x10^6 cells) per 1 ml ChIP reaction. If working with fewer cells, scale down the volumes proportionally.

16. Prepare the following ChIP reaction mixes for both the target antibody and negative control antibody[1]:

100 µl	Sheared Chromatin
x µl	ChIP-grade Antibody[2]
880 - x µl	**Chromatin Dilution Buffer**
10 µl	100X PIC[3]
10 µl	0.1 M PMSF
1 ml	**Final Volume**

Important! Be sure to set aside 10 µl of the sheared chromatin for use as a DNA input control. Keep DNA input control at 4 °C.

17. Incubate ChIP reactions 3 h to overnight at *4 °C* while rotating.

18. Add 15 µl **ZymoMag Protein A**[4] beads into the ChIP reactions and incubate for 1 h at *4 °C* while rotating.

19. Place tubes on a magnetic stand, allow the beads to cluster, and then remove and discard the supernatants.

20. Wash the Protein A bead/antibody/chromatin complexes by resuspending the beads in 1 ml each of the chilled buffers in the order listed below:
    Chromatin Wash Buffer I, 1st wash, 1 ml
    Chromatin Wash Buffer II, 2nd wash, 1ml
    Chromatin Wash Buffer III, 3rd wash, 1ml
    Incubate for 4 min while rotating between each wash followed by magnetic separation with careful removal of supernatants.

21. Resuspend beads in 500 µl **DNA Elution Buffer** *(do not confuse with 5X Chromatin Elution Buffer)* and transfer the bead suspension to a clean 1.5 ml microcentrifuge tube to avoid co-elution of protein that may be bound to the tube wall.

22. Place tubes on magnetic stand, allow the beads to cluster, and remove the supernatant.

Notes:

[1]A negative control antibody (e.g., immunoglobin G (IgG) antibody) is recommended in ChIP assays.

[2]Optimal results are typically obtained using 1 - 5 µg of antibody. However, the exact amount of antibody may vary according to the quality of the antibody.

[3]If required, sodium butyrate (10 mM final concentration) or other inhibitors can be added.

[4]Do not vortex, resuspend **ZymoMag Protein A** beads by pipetting.

SECTION 4: Elution and Reverse Cross-linking of ChIP DNA

23. Dilute the **5X Chromatin Elution Buffer** with water to obtain a 1X Chromatin Elution Buffer.
    Note: *If you notice a precipitate in the 5X Chromatin Elution Buffer, simply warm at 37°C until the precipitate is dissolved*.

24. Resuspend beads in 150 µl of 1X Chromatin Elution Buffer, gently mix by pipetting.

    Important! *Add 140 µl of 1X Chromatin Elution Buffer to 10 µl of the sheared chromatin (DNA input control) from Step 16.*

25. Add 6 µl **5 M NaCl** and mix by gently flicking the tube.

26. Incubate at *75 °C* for 5 min and centrifuge at ≥ 10,000 *x g* for 30 seconds.

27. Place tubes on magnetic stand, allow the beads to cluster, and then transfer eluates (ChIP DNA) into clean 1.5 ml microcentrifuge tubes.

28. Incubate at *65 °C* for 30 min.

29. Add 1 µl **Proteinase K** and incubate at *65 °C* for 90 min.

SECTION 5: ChIP DNA Purification

Buffer Preparation: *Before starting, add 24 ml 100% ethanol to the 6 ml* ***DNA Wash Buffer*** *concentrate to obtain working DNA Wash Buffer solution.*

30. Add 5 volumes of **ChIP DNA Binding Buffer** to each volume of DNA sample (5:1) and mix briefly (e.g. add 750 µl of **ChIP DNA Binding Buffer** to 150 µl ChIP DNA or input DNA sample).

31. Transfer mixture to a provided **Zymo-Spin™ IC Column** in a **Collection Tube**.

32. Centrifuge at ≥ 10,000 *x g* for 30 seconds. Discard the flow-through.

33. Add 200 µl **DNA Wash Buffer** to the column. Centrifuge at ≥ 10,000 *x g* for 30 seconds.

34. Repeat wash step (Step 33).

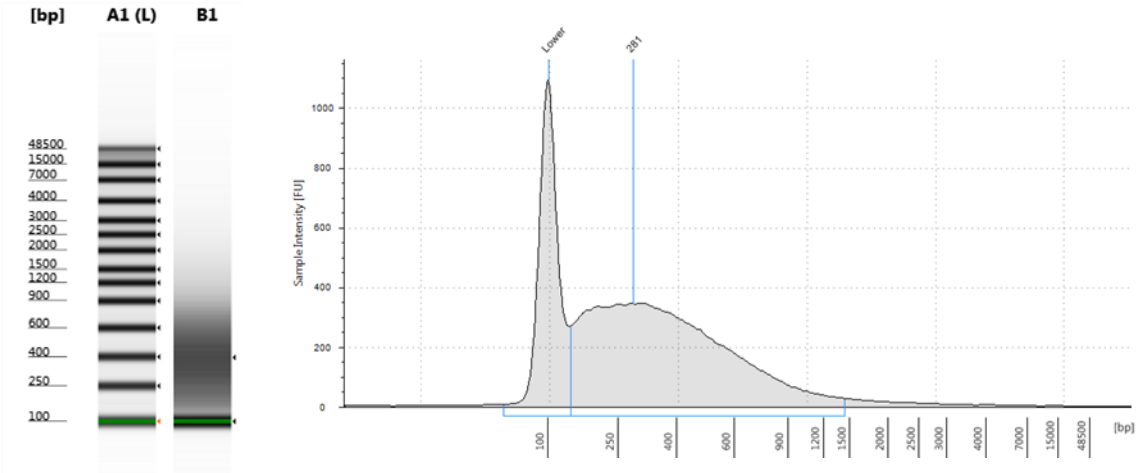
35. Transfer the column to a new 1.5 ml tube and add 8 µl **DNA Elution Buffer** directly to the column matrix. Let stand for 1 minute at room temperature and centrifuge at ≥ 10,000 *x g* for 30 seconds to elute the DNA.

36. Repeat elution (Step 35). Total elution volume for both ChIP DNA and input DNA samples is 16 µl.

37. Use immediately or store ChIP DNA at *-80 °C*.

APPENDIX A: Evaluation of Chromatin Shearing Efficiency

To assess chromatin shearing efficiency, remove 10 µl of sonicated chromatin from Step 15, reverse cross-link, purify sheared chromatin as described in SECTIONS 4 & 5, and analyze DNA size using agarose gel electrophoresis, Agilent Bioanalyzer or TapeStation. It is strongly recommended chromatin shearing be evaluated prior to proceeding with the ChIP protocol.



Chromatin Shearing Efficiency
Chromatin was prepared from HeLa cells as described in SECTIONS 1 & 2 of the Zymo-Spin™ ChIP Kit. Ten (10) µl of sheared chromatin was purified as described in SECTIONS 4 & 5 with 1 µl used in TapeStation analysis. The analysis indicates the DNA was effectively sheared with the majority of fragments migrating ≤ 600 bp (Lane B1).

APPENDIX B: Verification of ChIP DNA Enrichment using qPCR

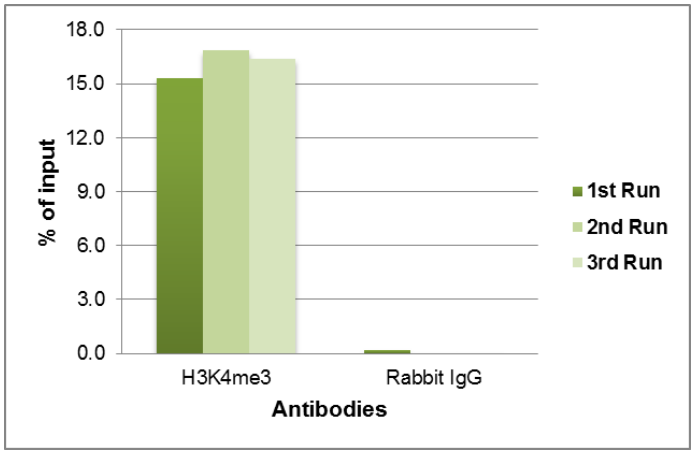
Following the ChIP procedure, enrichment of ChIP DNA can be verified using qPCR with primers specific for known control sequences. For example, the GAPDH promoter region can be used when comparing DNA immunoprecipitated with ChIP-grade anti-H3K4me3 versus that immunoprecipitated with a negative control antibody (e.g., rabbit IgG). **Quest*Taq*™ qPCR Premix (Cat. No. E2052, E2053)** can be used for real-time PCR according to the following conditions:

Prepare the reaction mix as follows:

10 µl	2X QuestTaq™ Premix
2 µl	GAPDH Primers (10 µM)
1 µl	ChIP DNA or input DNA
7 µl	ddH2O
20 µl	**Total Volume**

Suggested conditions for real-time PCR:

Initial Denaturation	95 °C 1 min
Denaturation	95 °C 30 sec
Annealing	55 °C 30 sec
Extension	72 °C 30 sec
	(30-45 Cycles)
Final Extension	72 °C 7 min



ChIP DNA Enrichment Verification by qPCR
Three independent ChIP assays were performed with HeLa cells and ChIP-grade anti-H3K4me3 and rabbit IgG using the Zymo-Spin™ ChIP Kit. The amount of ChIP DNA was determined using qPCR with primers specific to a region in the GAPDH promoter. ChIP DNA enrichment is graphed as % of input (i.e., the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

Ordering Information:

Product Descrption	Catalog No.	Kit Size
Zymo-Spin™ ChIP Kit	D5209 D5210	10 Preps. 25 Preps.

For Individual Sale	Catalog No.	Size
Nuclei Prep Buffer	D5220-2-30	30 ml
Chromatin Shearing Buffer	D5210-1-30	30 ml
Chromatin Dilution Buffer	D5210-2-30	30 ml
ZymoMag Protein A	M2001 M2001-2	200 µl 400 µl
Chromatin Wash Buffer I	D5210-3-30	30 ml
Chromatin Wash Buffer II	D5210-4-30	30 ml
Chromatin Wash Buffer III	D5210-5-30	30 ml
5X Chromatin Elution Buffer	D5210-6-10	10 ml
5 M NaCl	D5210-7-1	1 ml
Proteinase K	D3001-2-1	1 mg
ChIP DNA Binding Buffer	D5201-1-50	50 ml
DNA Wash Buffer	D4003-2-6	6 ml
DNA Elution Buffer	D3004-4-10 D3004-4-16	10 ml 16 ml
Zymo-Spin™ IC	C1004-25	25 columns
Collection Tubes (2 ml)	C1001-25	25 tubes

THE Epigenetics COMPANY™

Epigenetics Products From Zymo Research

Product	Description	Kit Size	Cat No. (Format)
	Chromatin Analysis		
ChIP DNA Clean & Concentrator™	Clean and concentrate DNA from any reaction or "crude" preparation in 2 min. A 6 µl minimum elution volume allows for highly concentrated DNA. Designed for samples containing up to 5 µg of DNA.	50 Preps. 50 Preps. 2x96 Preps. 4x96 Preps. 2x96 Preps. 4x96 Preps.	**D5201** (uncapped column) **D5205** (capped column) **D4017** (shallow-well plate) **D4018** (shallow-well plate) **D4023** (deep-well plate) **D4024** (deep-well plate)
EZ Nucleosomal DNA Prep Kit	The EZ Nucleosomal DNA Prep Kit is a streamlined procedure for the isolation of mammalian and yeast nucleosome-associated DNA. The kit includes procedures and reagents for: cell nuclei isolation, intact nuclei micrococcal nuclease digestion, and nucleosomal DNA purification. Non-nucleosomal DNA is specifically degraded using micrococcal nuclease and an optimized reaction buffer; while purification of "protected" nucleosome-associated DNA is performed using Zymo Research's proven Fast-Spin column technology.	20 Preps	**D5220** (spin column)
QuestTaq™ qPCR PreMix	QuestTaq™ qPCR PreMix contains all reagents necessary to perform unbiased quantitative PCR with DNA having 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), and glucosyl-5-hydroxymethylcytosine (g5hmC) composition. It features a unique DNA polymerase and optimized buffer system for amplification of DNA having modified/unmodified cytosines. It also includes an intense dsDNA-specific fluorescent dye (SYTO® 9) for sensitive/precise DNA detection.	50 Rxns. 200 Rxns.	**E2052** (premix) **E2053** (premix)
Zymo*Taq*™ DNA Polymerase	Zymo*Taq*™ "hot start" DNA Polymerase is specifically designed for the amplification of "difficult" DNA templates including: bisulfite-treated DNA for methylation detection. The product generates specific amplicons with little or no by-product formation. Available either as a single buffer premix or as a polymerase system with components provided separately.	50 Rxns. 200 Rxns. 50 Rxns. 200 Rxns.	**E2001** (system) **E2002** (system) **E2003** (premix) **E2004** (premix)
	Services		
Available for ***ChIP-Seq*** at http://www.zymoresearch.com/services or inquire at services@zymoresearch.com *powered by the latest Next-Gen sequencing technologies!*			