

# EZ Nucleosomal DNA Prep Kit

Cat. No. D5220 (20 preps)



Storage: -20 °C & Room Temperature

## Product Information

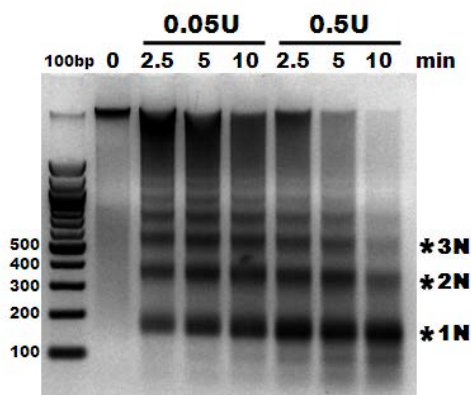
### Applications:

- For the isolation of nucleosome-associated DNA from *mammalian* and yeast cells.
- Ideal for use in nucleosome mapping studies.

### Description:

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. The result is pure nucleosomal DNA ready for analysis in less than 30 minutes!



**Mammalian Nucleosomal DNA Preparation:** Mammalian nuclei prepared as indicated by the *Mammalian Nuclei Prep Protocol* (see *opposing column*) was treated with 0.05 and 0.5 U (unit) micrococcal nuclease for the indicated times (min) at 25°C. DNA was subsequently resolved in a 2% agarose gel. 100 bp DNA ladder (Zymo Research Corp.). Asterisks (1N, 2N, 3N) represent mono-, di-, and tri-nucleosomal DNAs, respectively.

### Product Contents:

Component in D5220	Cat. No.	Amount	Storage
Micrococcal Nuclease (0.1U/μl)	D5220-1	10 U (100μl)	-20°C
Nuclei Prep Buffer	D5220-2	50 ml	RT
MN Digestion Buffer	D5220-3	50 ml	RT
5X MN Stop Buffer	D5220-4	6 ml	RT
DNA Binding Buffer	D4003-1-25	25 ml	RT
DNA Wash Buffer (concentrate)	D4003-2-6	6 ml	RT
DNA Elution Buffer	D3004-4-S	1 ml	RT
Zymo Spin IIC Column	C1011-20	20	RT
Collection Tube	C1001-20	20	RT

RT- Room Temperature

**Storage:** Store micrococcal nuclease at -20°C for up to 12 months. Avoid repeated freeze/thawing. Prolonged storage should be ≤-70 °C. All other reagents are stable at room temperature for up to 12 months.

**Micrococcal Nuclease Concentration:** 0.1 U/μl

### Nuclei Isolation Procedure

#### From Mammalian Cells (≤2.0x10<sup>7</sup> cells)

Note: a.) All steps carried out on ice or 4°C.  
b.) All buffers should be cold.  
c.) All spin steps are at 200 x g for 1 minute.

- Wash trypsinized cells with PBS and spin down.
- Decant supernatant and resuspend cells in 1 ml PBS.
- Transfer cells to 1.5 ml tube and spin in a microcentrifuge.
- Decant supernatant and add 1 ml Nuclei Prep Buffer to the cell pellet that can be resuspended by gentle pipetting or tube inversion.
- Incubate on ice for 5 minutes.
- Spin down nuclei and decant supernatant.
- Wash pelleted nuclei by adding 1 ml MN Digestion Buffer and inverting the tube gently 2-3 times.
- Spin down and decant supernatant.
- Repeat wash (Steps 7 & 8).

#### From Yeast Cells (≤7.5x10<sup>8</sup> cells)

Note: a.) All steps carried out on ice or 4°C.  
b.) All buffers should be cold.  
c.) All spin steps are at 200 x g for 1 minute.

- Add 1 ml Nuclei Prep Buffer to pelleted yeast spheroplasts and resuspend by gentle pipetting or tube inversion. (Yeast spheroplasting can easily be performed using Zymolyase from Zymo Research, Cat. Nos. E1004, E1005)
- Incubate on ice for 5 minutes.
- Spin down nuclei and decant supernatant.
- Wash pelleted nuclei by adding 1 ml MN Digestion Buffer and inverting the tube gently 2-3 times.
- Spin down and decant supernatant.
- Repeat wash (Steps 4 & 5).

### Treatment of Nuclei with Micrococcal Nuclease

A and B (below) are suggested parameters (i.e., U enzyme, time) for the treatment of mammalian or yeast nuclei with micrococcal nuclease. It is recommended that nuclease concentration and time be titrated for optimal results (see *opposing figure*).

#### A. Treatment of Mammalian Nuclei

Treat 1.5x10<sup>6</sup> mammalian nuclei with 0.05-0.5 units of micrococcal nuclease in 100 μl reaction volume at room temperature (25°C) for 5 minutes.

#### B. Treatment of Yeast Nuclei

Treat 2.5x10<sup>8</sup> yeast nuclei with 0.01-0.05 units of micrococcal nuclease in 100 μl reaction volume at room temperature (25°C) for 5 minutes.

### Protocol for Treatment of Nuclei with Micrococcal Nuclease

Note: a.) Reactions and buffers at room temperature (25°C)  
b.) If 5X MN Stop Buffer forms precipitate, incubate at 37 °C until it dissolves. Performance will not be adversely affected.

- Gently resuspend desired amount of nuclei in 100 μl MN Digestion Buffer (P1000 tip works best).
- Add a predetermined amount (units) (*reference above*) of micrococcal nuclease and mix by gently flicking the tube.
- Incubate at room temperature (~25°C) for 5 minutes.
- Stop the reaction by adding (1:5) 5X MN Stop Buffer to the reaction (e.g., 20 μl to 100 μl reaction).
- Vortex briefly... then proceed with *Nucleosomal DNA Purification* (see reverse side).

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The Beauty of Science is to Make Things Simple

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## Product Information

### Nucleosomal DNA Purification

#### Protocol for Nucleosomal DNA Purification

Note: a.) Before starting, add 24 ml 100% ethanol (OR 26 ml 95% ethanol) to 6 ml concentrate DNA Wash Buffer to obtain the final DNA Wash Buffer Solution

b.) All spin steps are performed at 10,000-13,000 x g.

1. Add a 5:1 ratio DNA Binding Buffer to the "stopped" reaction (e.g., 600 µl DNA Binding Buffer to a 120 µl reaction).
2. Mix well by vortexing.
3. Load into a Zymo-Spin™ IIC Column in a Collection Tube.
4. Spin for 30 seconds.
5. Decant flow through from the Collection Tube
6. Add 300 µl DNA Wash Buffer and spin for 30 seconds. Decant flow through.
7. Repeat wash (Step 6)
8. To remove any residual wash buffer, spin for 1 minute in the empty Collection Tube.
9. Transfer the spin column to a clean 1.5 ml microcentrifuge tube and add 30 µl DNA Elution Buffer or ddH<sub>2</sub>O directly to the column matrix and let stand at room temperature for ≥1 minute
10. Spin for 30 seconds to elute pure nucleosomal DNA.

#### Notes:

##### 1. Large-Scale Preparation

The main parameter that affects the efficiency of DNA digestion is the ratio of micrococcal nuclease to nuclei number, therefore, if using more nuclei, the units of enzyme may need to be increased. For larger-scale preparations or for final reaction volumes ≥800 µl, columns can be reloaded sequentially with the same sample to bind all of the nucleosomal DNA. Zymo-Spin™ IIC columns have a DNA binding capacity of ~25 µg/column.

##### 2. Nuclei Isolation

Nuclei isolation should be carried out on ice using cold buffers. Pelleted nuclei are typically white and will often clump together. Gentle resuspension is accomplished by pipetting with a wide bore pipette tip (P1000) or by gentle flicking of the tube. The efficiency of the isolation may be assessed by staining with trypan blue. Nuclei will stain blue but not when in intact cells. For most nuclei isolations, the Nuclei Prep Buffer is suitable but nuclei from some cell lines may be sensitive to the detergent concentration in the buffer. Should this be the case, it is recommended that the Nuclei Prep Buffer be diluted with MN Digestion Buffer at a 1:1 ratio (Nuclei Prep Buffer:MN Digestion Buffer) prior to Nuclei Isolation.

##### 3. Micrococcal Nuclease Treatment

While incubating nuclei with micrococcal nuclease in MN Digestion Buffer, it is normal for the reaction to form a white precipitate in addition to the nuclei that are present. This will not affect the quality of the nucleosomal DNA preparation in any way.

#### Related Epigenetics Products:

Product Name	Size	Cat. No.
<b>BISULFITE TREATMENT OF DNA</b>		
EZ DNA Methylation™ Kit	50 rxns.	D5001
	200 rxns.	D5002
	2 x 96 rxns.	D5003
	2 x 96 rxns.	D5004
EZ DNA Methylation-Gold™ Kit	50 rxns.	D5005
	200 rxns.	D5006
	2 x 96 rxns.	D5007
	2 x 96 rxns.	D5008

EZ DNA Methylation-Direct™ Kit	50 rxns. 200 rxns. 2 x 96 rxns. 2 x 96 rxns.	D5020 D5021 D5022 D5023
EZ DNA Methylation-Startup™ Kit	50 rxns.	D5024
EZ Bisulfite DNA Clean-up Kit™	50 preps. 200 preps. 2 x 96 preps. 2 x 96 preps.	D5025 D5026 D5027 D5028
<b>METHYLATED/NON-METHYLATED DNA STANDARDS</b>		
Universal Methylated DNA Standard	1 set	D5010
Universal Methylated Human DNA Standard	1 set	D5011
Universal Methylated Mouse DNA Standard	1 set	D5012
Human Methylated and Non-methylated DNA Set	1 set	D5014
Human HCT116 DKO Non-methylated DNA Standard	5 µg	D5014-1
Human HCT116 DKO Methylated DNA Standard	5 µg	D5014-2
Bisulfite Converted Universal Methylated Human DNA Standard	1 set	D5015
<i>E. coli</i> Non-methylated Genomic DNA	5 µg	D5016
<b>AMPLIFICATION OF BISULFITE CONVERTED DNA</b>		
ZymoTaq™ DNA Polymerase	50 rxns. 200 rxns.	E2001 E2002
ZymoTaq™ PreMix (2X concentrated)	50 rxns. 200 rxns.	E2003 E2004
<b>ANTIBODIES &amp; IMMUNOPRECIPITATION</b>		
Methylated-DNA IP Kit	10 preps.	D5101
ChIP DNA Clean & Concentrator™	50 preps. 50 preps.	D5201 D5205
Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)	50 µg 200 µg	A3001-50 A3001-200
<b>METHYLTRANSFERASES</b>		
CpG Methylase (M.SssI)	200 U 400 U	E2010 E2011
	GpC Methylase (M.CviPI)	200 U 1000 U
<b>DNA FRAGMENTATION</b>		
DNA Degradase™	500 U 2000 U	E2016 E2017
	DNA Degradase Plus™	250 U 1000 U
DNA Shearase™		50 U
	200 U	E2018-200
	50 U & DCC™	E2019-50
	200 U & DCC™	E2019-200
<b>NUCLEOSOME MAPPING</b>		
EZ Nucleosomal DNA Prep Kit	20 preps	D5220
<b>5-HYDROXYMETHYLCYTOSINE</b>		
5-Hydroxymethylcytosine DNA	5 µg	D5400
5-Methylcytosine & 5-Hydroxymethylcytosine DNA Standard Set	1 set	D5405

#### Trademarks and Disclaimers:

™ Trademarks of Zymo Research Corporation.

This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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 maximal performance and reliability.

For Technical Assistance, please contact 1-888-882-9682 or E-mail [tech@zymoresearch.com](mailto:tech@zymoresearch.com). Satisfaction of all Zymo Research products is guaranteed. If you are not satisfied with this product please call 1-888-882-9682.

Version 1.0.2

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