EZ Nucleosomal DNA Prep Kit

Cat. No. D5220 (20 preps)

Storage: -20 °C & Room Temperature

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Product Information

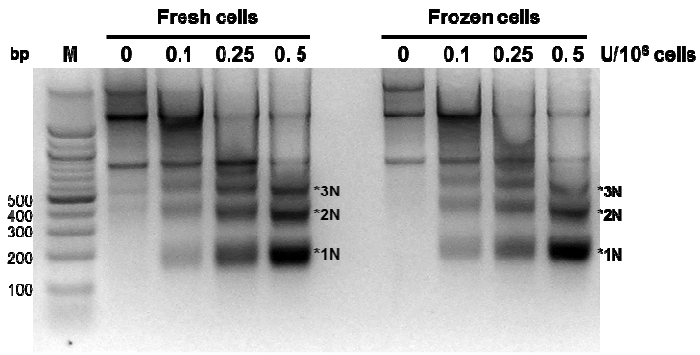
Applications:

- For the isolation of nucleosome-associated DNA from fresh or frozen cells.
- Ideal for use in nucleosome mapping studies.
- Contains a newly developed enzyme - **Atlantis dsDNase** that replaces conventional micrococcal nuclease for nucleosomal DNA preparation.
- **Atlantis dsDNase** digestion yields homogenous populations of core nucleosomes.

Description:

The EZ Nucleosomal DNA Prep Kit is a streamlined procedure for the isolation of nucleosome-associated DNA. The kit includes reagents/procedures for: cell nuclei isolation, intact nuclei enzymatic digestion, and nucleosomal DNA purification. This kit includes two different enzymes for nucleosomal DNA preparation: Atlantis dsDNase and micrococcal nuclease.

Atlantis dsDNase is a double-strand DNA specific endonuclease that cleaves phosphodiester bonds in DNA to yield oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. Atlantis dsDNase digestion yields very homogeneous populations of core nucleosomes and purification of the 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 *45 minutes*!



Mammalian Nucleosomal DNA Preparation: Mammalian nuclei prepared as indicated by the *Mammalian Nuclei Prep Protocol (see next page*) was treated with 0.1 U, 0.25 U, and 0.5 U (unit) Atlantis dsDNase for the 20 min at 42°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.

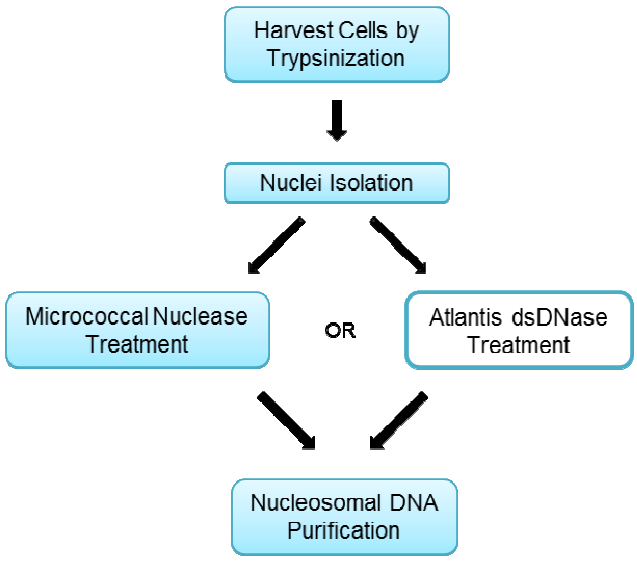
Product Contents:

Component in D5220	Cat. No.	Amount	Storage
Atlantis dsDNase (0.1 U/µl)	E2030	12.5 U (125 µl)	-20°C
Atlantis Digestion Buffer	E2030-1	50 ml	RT
Micrococcal Nuclease (0.1 U/µl)	D5220-1	10 U (100 µl)	-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 Atlantis dsDNase and 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.

Outline of the Nucleosomal DNA Prep Kit



Suggested parameters:

	Cell Density	Atlantis dsDNase	Micrococcal Nuclease
Mammalian cells	$1x10^6$	0.25-0.5 U	0.05-0.5 U

The following protocol is based on samples of approximately $1x10^6$ cells.

Nuclei Isolation Procedure

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

1. Wash trypsinized cells with PBS twice and pellet the cells by centrifugation. ***These washing steps are critical for the removal of excess EDTA present in the cell culture medium and trypsin.***
2. Discard supernatant completely and resuspend cell pellet in 1 ml PBS.
3. Transfer cells to 1.5 ml tube and centrifuge.
4. Discard supernatant completely and add 100 µl Nuclei Prep Buffer to the cell pellet and resuspend by gentle pipetting or tube inversion.
5. Incubate on ice for 5 minutes.
6. Pellet cell nuclei by centrifugation and discard supernatant.
7. Wash pelleted nuclei by adding 100 µl Atlantis Digestion Buffer and inverting the tube gently 2-3 times.
8. Centrifuge and discard supernatant.
9. Repeat wash (Steps 7 & 8).
10. Proceed with either *Atlantis dsDNase* or *Micrococcal Nuclease Treatment.*

Enzymatic Treatment

Note: a.) Only one enzyme (either Atlantis dsDNase or micrococcal nuclease) is needed for enzymatic treatment.
b.) If 5X MN Stop Buffer forms a precipitate, incubate at 37 °C until dissolved. Performance will not be affected.

Atlantis dsDNase Treatment

1. Gently resuspend cell nuclei in 100 µl of Atlantis Digestion Buffer.
2. Add 0.25–0.5 U of Atlantis dsDNase and mix by gently flicking the tube.
3. Incubate at 42°C for 20 minutes.
4. Stop the reaction by adding (1:5) 5X MN Stop Buffer to the reaction (e.g., 20 µl to 100 µl reaction).
5. Vortex briefly and proceed with *Nucleosomal DNA Purification*.

Micrococcal Nuclease Treatment

1. Gently resuspend cell nuclei in 100 µl of MN Digestion Buffer.
2. Add 0.01–0.5 units of micrococcal nuclease and mix by gently flicking the tube.
3. Incubate at room temperature (~25°C) for 5 minutes.
4. Stop the reaction by adding (1:5) 5X MN Stop Buffer to the reaction (e.g., 20 µl to 100 µl reaction).
5. Vortex briefly and proceed with *Nucleosomal DNA Purification*.

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 centrifuge 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. Discard flow through from the Collection Tube.
6. Add 300 µl DNA Wash Buffer and spin for 30 seconds. Discard 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_2O 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 enzyme 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 Atlantis dsDNase Digestion Buffer or MN Digestion Buffer at a 1:1 ratio (Nuclei Prep Buffer : Digestion Buffer) prior to Nuclei Isolation.

3. Enzymatic Treatment

While incubating nuclei with Atlantis dsDNase or micrococcal nuclease in 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.

Related Products:

Product Name	Size	Cat. No.
Atlantis dsDNA Shearase™	50 U 200 U	E2018-50 E2018-200
Atlantis dsDNA Shearase & DCC-5™	50 preps 200 preps	E2019-50 E2019-200