Instruction Manual Version 2 - 01.14

DNA Elution Module

Cat. No. **C01010120** (mc-magme-002)

diagenode

A Hologic Company

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Content

MANUAL

Introduction

The DNA elution module allows the elution of chromatin and DNA from immunoprecipitated material after ChIP and MeDIP respectively. Afterwards, you can proceed to the DNA purification using columns or carrying out a phenol/chloroform/isomayl alcohol extraction.

Kit Material

Kit Content

Component	Comments	Quantity	Storage
Buffer D	-	6 ml	4°C
Buffer E	Detergent included. Need to be placed at room temperature 1 hour before use	1 ml	4°C
Buffer F	Salt included	500 µl	4°C
meDNA-IP TE	Ion chelator included	5 ml	4°C
meDNA-IP co-precipitant	-	100 µl	- 20°C
meDNA-IP precipitant	-	1 ml	4°C

Reagents and Equipment Not Provided

- Qiaquick PCR purification kit (option #1)
- Phenol-Chloroform Isoamyl (option #2)
- Chloroform Isoamyl (Option #2)
- Ethanol 100 % and 70% (Option #2)

Protocol

Part I. DNA purification after Methylated DNA IP (MeDIP Kit; C02010010)

Option #1: Elution followed by purification using QIAquick PCR columns (QIAGEN # 28106).

1. Take the input samples, centrifuge briefly and from now onwards treat the input DNA samples and IP samples in parallel.
   - input samples (STEP 6- Point 1.).
   - Methyl DNA IP samples (STEP 6- Point 1.).

2. Prepare the complete elution buffer by mixing Buffer D, E and F as follows.
   - The right column gives the volume of complete elution buffer to prepare for 2 IPs and 10% input. (Note that the final volume includes about 10% excess).

Table 8. Complete elution buffer

Complete elution buffer	1 Methyl DNA IP	2 IPs + input
Buffer D	103.50 µl	335 µl
Buffer E	11.50 µl	37 µl
Buffer F	5.00 µl	16 µl
Total volume	**120.00 µl**	**388 µl**

3. Add 120 µl of freshly prepared complete elution buffer to the bead pellets (the Methyl DNA IP samples).

4. Add 120 µl of freshly prepared complete elution buffer to the input samples.

5. Incubate in a thermo-shaker for 10 minutes at 65ºC at 1,000 to 1,300 rpm.

6. Place tubes in the DiaMag02 (magnetic rack; cat#kch-816-001), wait for 1 minute.

7. Remove the supernatant and transfer it to a new 1.5 ml tube. This is the DNA to be purified.

8. Then, use the QIAquick instructions. In brief: add 600 µl PB, vortex, and apply sample to column, centrifuge at 13,000 rpm for 1 minute (possibility to combine the two methyl DNA IP duplicate samples per column).

9. Wash with 700 µl PE, centrifuge at 13,000 rpm for one minute, get rid of the flow-through.

10. Spin at 13,000 rpm for one minute.

11. Elute with 50 µl TE.
    - Make sure that the TE buffer is at pH 8.00. The yield will decrease if the pH is lower.

12. Incubate at room temperature for 1 minute.

13. Centrifuge at 13,000 rpm for one minute.

At this stage, you obtain purified DNA from: 1/ the sheared DNA (input sample(s)) and 2/ DNA that was isolated by Methyl DNA IP (Methyl DNA IP sample(s)). Proceed to STEP 3- qPCR or other downstream DNA analysis.

Option #2: Elution followed by Phenol / Chloroform / Isoamyl alcohol extraction.

1. Take the input samples, centrifuge briefly and from now onwards treat the input DNA samples and IP samples in parallel.
   - Input samples (STEP 1- Point 6.).
   - IP samples (STEP 1- Point 21.).

2. Prepare the complete elution buffer by mixing Buffer D, E and F as follows.
   - The right column gives the volume of complete elution buffer to prepare for 2 IPs and 10% input. (Note that the final volume includes about 10% excess).

Table 8. Complete elution buffer

Complete elution buffer	1 Methyl DNA IP	2 IPs + input
Buffer D	360.00 µl	1188.00 µl
Buffer E	40.00 µl	132.00 µl
Buffer F	16.00 µl	53.00 µl
Total volume	**416.00 µl**	**1,373.00 µl**

3. Add 416 µl of freshly prepared complete elution buffer to the bead pellets (the Methyl DNA IP samples).

4. Add 416 µl of freshly prepared complete elution buffer to the input samples.

5. Incubate in a thermo-shaker for 10 minutes at 65ºC at 1,000 to 1,300 rpm.

6. Place tubes in the DiaMag02 (magnetic rack; cat#kch-816-001), wait for 1 minute.

7. Remove the supernatant and transfer it to a new 1.5 ml tube. This is the DNA to be purified.

8. Cool down samples to room temperature, add 1 volume of phenol/chloroform/isoamyl alcohol (25:24:1) (416 µl). Incubate the samples at RT for 10 minutes on a rotating wheel before centrifugation. Use gentle rotation.

9. Centrifuge for 2 minutes at 14,000x g (13,000 rpm) at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

10. Add 1 volume of chloroform/isoamyl alcohol (24:1) (416 µl).
    Incubate the samples at RT for 10 minutes on a rotating wheel before centrifugation. Use gentle rotation.
    Meanwhile, thaw the DNA co-precipitant on ice.

11. Centrifuge for 2 minutes at 14,000x g (13,000 rpm) at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

12. Per tube: add 5 µl of the provided DNA-IP co-precipitant and 40 µl of the DNA-IP precipitant. Then, add 1 ml of ice-cold 100% ethanol. Mix well. Leave at -20°C for minimum 30 minutes.

13. Centrifuge for 25 minutes at 14,000x g (13,000 rpm) at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70% ethanol to the pellet.

14. Centrifuge for 10 minutes at 14,000x g (13,000 rpm) at 4°C. Carefully remove the supernatant, leave tubes opened for 30 minutes at room temperature to evaporate the remaining ethanol. The pellets are: 1/ DNA that was purified from the sheared DNA (input sample(s)) and 2/ DNA that was isolated by IP (Methyl DNA IP samples).
    Avoid leaving ethanol on tube walls. The sheared DNA that is taken as input sample must correspond to the same preparation of sheared DNA used in the IP assay.

15. Add of 50 µl TE or water to the IP and input samples.
Suspend the DNA evenly: place the tubes in a shaker for 30 minutes at 12,000 rpm at room temperature to dissolve the pellets.

At this stage, you obtain purified DNA from: 1/ the sheared DNA (input sample(s)) and 2/ DNA that was isolated by Methyl DNA IP (Methyl DNA IP sample(s)). Proceed to downstream analysis, such as qPCR, sequencing ...

MANUAL

Part II. DNA purification after Chromatin Immunoprecipitation (ChIP)

Option #1: Elution followed by purification using QIAquick PCR columns (QIAGEN # 28106).

1. Take the input samples, centrifuge briefly and from now onwards treat the input DNA samples and IP samples in parallel.
   - Input samples
   - IP samples

2. Prepare the complete elution buffer by mixing Buffer D, E and F as follows.
   - The right column gives the volume of complete elution buffer to prepare for 2 IPs and input. Note that the final volume includes about 10% excess.
   - If using more than 10% input, adjust final volumes accordingly.

Table7. Complete elution buffer

Complete elution buffer	1 IP	2 IPs + input
Buffer D	103.50 µl	335.00 µl
Buffer E	11.50 µl	37.00 µl
Buffer F	5.00 µl	16.00 µl
Total volume	**120.00 µl**	**388.00 µl**

3. Add 120 µl of freshly prepared complete elution buffer to the bead pellets (the ChIP samples).

4. Add 120 µl of freshly prepared complete elution buffer to the input samples.

5. Incubate in a thermo-shaker for minimum 4 hours to maximum O.N. at 65ºC at 1,000 to 1,300 rpm.

6. Place tubes in the DiaMag02 (magnetic rack; cat#kch-816-001), wait for 1 minute.

7. Remove the supernatant and transfer it to a new 1.5 ml tube. This is the DNA to be purified.

8. Then, use the QIAquick instructions. In brief: add 600 µl PB, vortex, and apply sample to column, centrifuge at 13,000 rpm for 1 minute (possibility to combine the two IP duplicate samples per column).

9. Wash with 700 µl PE, centrifuge at 13,000 rpm for one minute, get rid of the flowthrough.

10. Spin at 13,000 rpm for one minute.

11. Elute with 50 µl TE. Make sure that the TE buffer is at pH 8.00. The yield will decrease if the pH is lower.

12. Incubate at room temperature for 1 minute.

13. Centrifuge at 13,000 rpm for one minute.

Option #2: Elution followed by Phenol/Chloroform/Isoamyl alcohol extraction.

1. Take the input samples, centrifuge briefly and from now onwards treat the input DNA samples and IP samples in parallel.
   - Input samples
   - IP samples

2. Prepare the complete elution buffer by mixing Buffer D, E and F as follows.
   - The right column gives the volume of complete elution buffer to prepare for 2 IPs and input. Note that in the table, the final volume includes about 10% excess.
   - If using more than 10% input, adjust final volumes accordingly.

Table7. Complete elution buffer

Complete elution buffer	1 IP	2 IPs + input
Buffer D	360.00 µl	1,188.00 µl
Buffer E	40.00 µl	132.00 µl
Buffer F	16.00 µl	53.00 µl
Total volume	**416.00 µl**	**1,373.00 µl**

3. Add 416 µl of freshly prepared complete elution buffer to the bead pellets (the ChIP samples).

4. Add 416 µl of freshly prepared complete elution buffer to the input samples.

5. Incubate in a thermo-shaker for minimum 4 hours to maximum O.N. at 65ºC at 1,000 to 1,300 rpm.

6. Place tubes in the DiaMag02 (magnetic rack; cat#kch-816-001), wait for 1 minute.

7. Remove the supernatant and transfer it to a new 1.5 ml tube. This is the DNA to be purified.

8. Cool down samples to room temperature, add 1 volume of phenol/chloroform/isoamyl alcohol (25:24:1).
   - It is possible to incubate the samples at RT for 10 minutes on a rotating wheel before centrifugation. Use gentle rotation.

9. Centrifuge for 2 minutes at 14,000x g (13,000 rpm) at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

10. Add 1 volume of chloroform/isoamyl alcohol (24:1).
    - It is possible to incubate the samples at RT for 10 minutes on a rotating wheel before centrifugation. Use gentle rotation.
    - Meanwhile, thaw the DNA co-precipitant on ice.

11. Centrifuge for 2 minutes at 14,000x g (13,000 rpm) at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

12. Per tube: add 5 µl of the provided DNA-IP co-precipitant and 40 µl of the DNA-IP precipitant. Then, add 1 ml of ice-cold 100% ethanol. Mix well. Leave at -20°C for 30 minutes.

11. Centrifuge for 25 minutes at 14,000x g (13,000 rpm) at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70% ethanol to the pellet.

12. Centrifuge for 10 minutes at 14,000x g (13,000 rpm) at 4°C. Carefully remove the supernatant, leave tubes opened for 30 minutes at room temperature to evaporate the remaining ethanol. The pellets are: 1/ DNA that was purified from the sheared chromatin (input sample(s)) and 2/ DNA that was isolated by IP (ChIP samples).
    - Avoid leaving ethanol on tube walls.
    - The sheared chromatin that is taken as input sample must correspond to the same preparation of sheared chromatin used in the IP assay.

13. Add of 50 µl TE or water to the IP and input samples.
    - Suspend the DNA evenly: place the tubes in a shaker for 30 minutes at 12,000 rpm at room temperature to dissolve the pellets.

At this stage, you obtain purified DNA from: 1/ the sheared chromatin (input sample(s)) and 2/ DNA that was isolated by ChIP (ChIP sample(s)). Proceed to downstream analysis, such as qPCR, sequencing, ….

Ordering information

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MANUAL

Diagenode is recognized as the "Best-in-class" Epigenetic Antibody Company by EU programs, Academic labs and Biotech companies.

Research continues to demonstrate the importance of histone modifications and how they can regulate gene expression. These include their interplay with histone variants, DNA methylation, transcription factors, RNA components and ATP-dependent chromatin remodeling or assembly factors.

There are tremendous opportunities offered by the use of immuno-techniques like immunoprecipitation assays (especially in combination with DNA microarrays and High Throughput sequencing technologies). At Diagenode we know that a successful immunoprecipitation requires an effective, specific and high quality antibody. Therefore we focus our efforts on ensuring all of our antibodies meet our stringent quality control standards.

Diagenode is involved in several European programs; often requiring us to produce high quality antibodies for the epigenetic field. This has allowed us to develop our antibody production and characterization procedures together with academic researchers. Therefore guaranteeing our antibodies meet the standards required by you; the researcher.

Selection of our Antibodies

The production of our antibodies is selected through a number of sources including:

→ Direct collaborations (including European programs)
→ Customer suggestions (licensing)
→ Research focus areas
→ Conferences

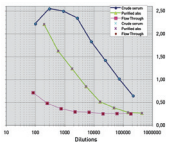
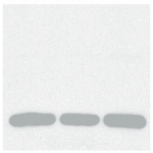
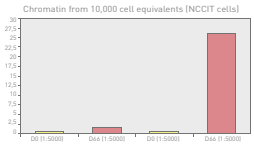
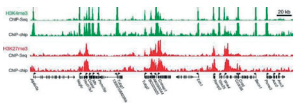
The majority of our antibodies are developed and characterized in-house. These are complemented with high quality antibodies sourced from academic laboratories or primary manufacturers.

Antibody production, characterization and quality controls at Diagenode

Below is an outline of our robust antibody production procedures, which have been developed in collaboration with our academic partners. Most of our antibodies are directed against epigenetic specific targets; including histone modifications, chromatin modifying and/or interacting proteins as well as transcription factors. Diagenode's goal is to characterize every batch of antibody using established QC procedures and therefore maximizing the reproducibility of our antibody between batches.

1. **Peptide design –** Our R&D department has developed techniques to increase the immunogenicity of our peptides to increase the titre and specificity of our antibodies.
2. **Titre analysis –** We analyse the immune-response in the crude sera by performing pepide-ELISA.
3. **Affinity purification –** This is performed using the immunizing peptide.
4. **Characterization –** The characterization of an antibody is the most important step of this process and we will commonly test using Peptide ELISA, Western Blot, Immunofluorescence and / or Dot Blot techniques.
5. **ChIP-Grade validation** – Specific antibodies will be characterized using Chromatin Immunoprecipitation techniques with all our data available on our antibody datasheets.
6. **Diagenode Next Generation Characterization** – Specific antibodies will be characterized using our in-house Illumina Illumina Instrument (ChIP-seq) and / or through collaborative agreements using microarray approaches (ChIP-on-chip).

QC flowchart (step by step)

CRUDE SERA		SINGLE AFFINITY PURIFIED
• Immune response • Screening different bleeds, comparison		• QC purification • Checking response in comparison with • crude serum • Fine titration
	Dot Blot	
• Specificity • Screening targeted cross reactions		• Specificity • Screening targeted cross reactions
	Western Blot	
• Testing global specificity		• Testing global specificity
• Immunofluorescence if applicable		• Immunofluorescence & ELISA inhibition if applicable
	Chromatin IP	
• Chromatin IP • Screening of dilutions of different bleeds		• Chromatin IP • Checking efficiency and quality of crude sera / purified Ab
	ChIP-chip / ChIP-seq	
		

Antibodies that have been consistent in ChIP are applied in the technique of
ChIP-on-chip and ChIP-seq through collaborative aggreement.

MANUAL

Bringing it all together: Diagenode's ChIP-seq workflow

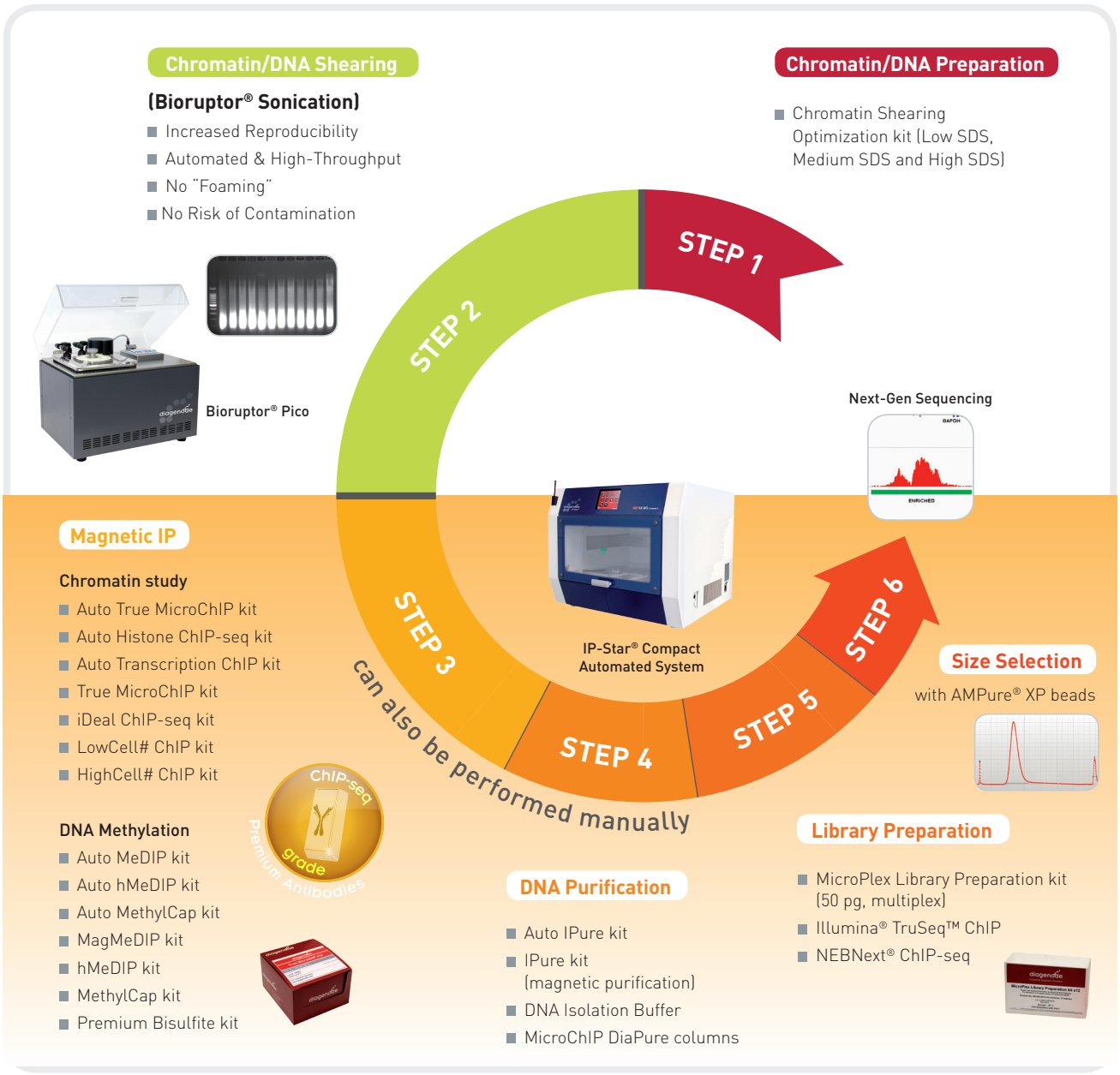


Figure 1. Diagenode provides a full suite of manual and automated solutions for ChIP experiments.

For Step 1, we offer products to isolate nuclei and chromatin. Step 2 describes reproducible sample shearing with the Bioruptor® product line. In Step 3 and Step 4, the Diagenode IP-Star® Compact provides error-free, walk-away automation for all your immunoprecipitation and antibody capture needs.

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