Instruction Manual Version 4 - 12.15

IPure kit

Magnetic DNA Purification kit for epigenetic applications

Cat. No. **C03010011** (AL-100-0024)

diagenode

A Hologic Company

Content

MANUAL

Introduction

Diagenode's IPure kit is the only DNA purification kit using magnetic beads, that is specifically optimized for extracting DNA from ChIP and MeDIP (Chromatin IP and Methylated DNA IP).

It's a simple and straightforward protocol that delivers pure DNA ready for any downstream application (e.g. next generation sequencing). This approach guarantees a minimal loss of DNA and reaches significantly higher yields than a column purification (see results pag). Comparing to phenol-chloroform extraction, the IPure technology has the advantage of being nontoxic and much easier to be carried out on multiple samples. The use of the magnetic beads allows for a clear separation of DNA and increases therefore the reproducibility of your DNA purification.

Two types of magnetic racks can be used:

DiaMag1.5 - magnetic rack
Cat. No. kch-716-015

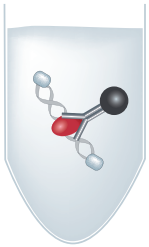
- Holds 12x standard 1.5 ml tubes
- Working volume: 25-1000 µl

DiaMag02 - magnetic rack
Cat. No. kch-816-001

- Holds 16x standard 0.2 ml tubes
- Working volume: 25-200 µl

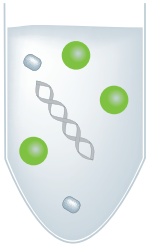
Method Overview

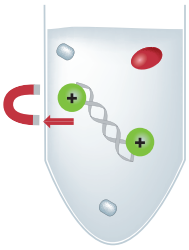
IPure after ChIP



STEP 1. Chromatin reverse cross-linking and elution

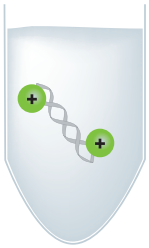
Chromatin is decrosslinked and eluted from **beads** (magnetic or agarose) which are discarded.
Magnetic beads for purification are added.





STEP 2. DNA binding

Magnetic beads acquire positive charge to bind the negatively charged phosphate backbone of DNA.
DNA-bead complex is separated using a magnet.



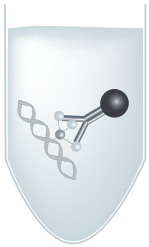
STEP 3. Washes

Proteins and remaining buffer are washed away.

STEP 4. DNA Elution

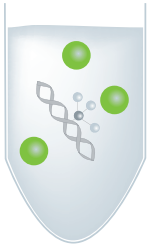
DNA is eluted from magnetic beads, which are discarded.
Purified DNA is ready for any downstream application:
- Next generation sequencing
- qPCR
- Amplification
- Microarray

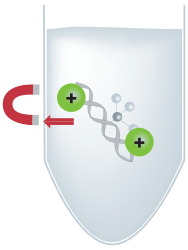
IPure after MeDIP



STEP 1. DNA Elution

DNA is eluted from **beads** (magnetic or agarose).
Magnetic beads for purification are added.





STEP 2. DNA binding

Magnetic Beads acquire positive charge to bind the negatively charged phosphate backbone of DNA.
DNA-bead complex is separated using a magnet.

STEP 3. Washes

Remaining buffer is washed away.

STEP 4. DNA Elution

DNA is eluted from magnetic beads, which are discarded.
Purified DNA is ready for any downstream application:
- Next generation sequencing
- qPCR
- Amplification
- Microarray

Kit Content

The kit content is sufficient to perform 24 reactions.

IPure kit		
Description	Quantity (x24)	Storage
200 µl tube strips (8 tubes/strip) + cap strips	4 pc	Room temperature
Buffer A	3.5 ml	4°C
Buffer B	125 µl	4°C
Wash buffer 1 w/o isopropanol	2 ml	4°C
Wash buffer 2 w/o isopropanol	2 ml	4°C
Buffer C	4 ml	4°C
Magnetic beads	400 µl	4°C
Carrier*	55 µl	-20°C

*This product is shipped at 4°C. Store it at -20°C upon arrival. This is an optimized buffer (NOT CARRIER DNA).

Related products		
Description	Cat. No.	Format
DiaMag02 - magnetic rack	kch-816-001	1 pc
DiaMag1.5 - magnetic rack	kch-716-015	1 pc
200 µl tube strips (8 tubes/strip) + cap strips	WA-002-0120	120 pc
LowCell# ChIP kit	kch-maglow-016	16 rxns
Auto ChIP kit x16	AB-Auto01-0016	16 rxns
Auto ChIP kit x100	AB-Auto01-0100	100 rxns
MagMeDIP kit x10	mc-magme-A10	10 rxns
MagMeDIP kit x48	mc-magme-048	48 rxns
MeDIP kit	mc-green-003	10 rxns
Auto MeDIP kit x16	AF-Auto01-0016	16 rxns
Auto MeDIP kit x100	AF-Auto01-0100	100 rxns

Required materials not provided

- DiaMag02 - magnetic rack (kch-816-001) / DiaMag1.5 - magnetic rack (kch-716-015)
- Microcentrifuge for 0.2 ml tubes or for 1.5 ml tubes with corresponding adaptor.
- 100% isopropanol
- Sodium Acetate
- 70% ethanol
- 100% ethanol
- DNase-free water

Time table

IPure after ChIP (Chromatin Immunoprecipitation)		
STEP 1	Chromatin reverse crosslinking and elution	4 h (or overnight)
STEP 2	DNA binding	1 h
STEP 3	Washes	10 min
STEP 4	DNA elution (option I / option II)*	30 min / 2 h 30min
Total time		**5 h 40 min / 7 h 40 min**

IPure after MeDIP (Methylated DNA Immunoprecipitation)		
STEP 1	DNA elution	30 min
STEP 2	DNA binding	1 h
STEP 3	Washes	10 min
STEP 4	DNA elution (option I / option II)*	30 min / 2 h 30min
Total time		**2 h 10 min / 4h 10 min**

*option I: two elutions in 25 µl (see page 14)
option II: two elutions in 75 µl followed by ethanol precipitation (see page 14)

MANUAL

Protocol

Short protocol

After the last wash of immunoprecipitated material (ChIP or MeDIP), discard the last traces of wash buffer and use the pellet of beads (8-tube strip) for STEP 1a (ChIP) or STEP 1b (MeDIP).

STEP 1a: Chromatin reverse crosslinking and elution (ChIP)

1. Prepare the Elution buffer by mixing Buffer A and B as follows:

Elution buffer	1 rxn
Buffer A	115.4 µl
Buffer B	4.6 µl
Total volume	**120 µl**

2. Add 100 µl of Elution buffer to the bead pellets (tube strip).
3. Thaw your input sample (1.5 ml tube), and perform a pulse spin. Add 90 µl of Elution buffer and 10 µl of input sample to a new 200 µl tube (8 tube-strip).
4. Incubate samples and input for 4 hours (or overnight) at 65°C on a thermomixer, with continuous shaking.
5. Spin the 8-tube strip and place it into the DiaMag02 or DiaMag1.5 (magnetic rack). After 1 minute, transfer the supernatants to a new labelled 8-tube strip. Keep the samples on ice.

STEP 1b: DNA elution (MeDIP)

1. Prepare the Elution buffer by mixing Buffer A and B as follows:

Elution buffers	1 rxn
Buffer A	115.4 µl
Buffer B	4.6 µl
Total volume	**120 µl**

2. Add 50 µl of Elution buffer to the bead pellets (tube strip).
3. Thaw your input sample (1.5 ml tube), and perform a pulse spin. Add 92.5 µl of Elution buffer and 7.5 µl of input sample to a new 200 µl tube (8-tube strip).
4. Incubate samples and input DNA for 15 min at room temperature on a rotating wheel (40 rpm).
5. Spin the 8-tube strip and place it into the DiaMag02 or DiaMag1.5 (magnetic rack). After 1 minute, transfer the supernatants to a new labelled 8-tube strip.
6. Repeat the incubation of the bead pellets for 15 min at room temperature on a rotating wheel (40 rpm) in 50 µl elution buffer.
7. Spin the 8-tube strip. Place the 8-tube strips into the DiaMag02 or DiaMag1.5 containing the 50 µl IP samples, wait 1 minute and transfer the supernatants to the new labelled 8-tube strip to pool with the corresponding IP samples.

STEP 2: DNA binding (ChIP & MeDIP)

1. Add **2 µl of carrier** to each IP and input sample. Vortex briefly and perform a short spin.
2. Add **100 µl of 100% isopropanol** to each IP and input sample. Vortex briefly and perform a short spin.

Following the addition of isopropanol the solution may become cloudy. This is not detrimental to your experiment and will not influence the quality or quantity of your purified DNA.

3. Resuspend the provided Magnetic beads and transfer 15 µl to each IP and input sample.
4. Incubate IP and input samples for 1 hour at room temperature on a rotating wheel (40 rpm).

STEP 3: Washes (ChIP & MeDIP)

1. Prepare the Wash buffer 1 containing 50% isopropanol for 24 reactions:

Wash buffer 1	24 rxns	100 rxns
Wash buffer 1 w/o isopropanol	2 ml	8 ml
Isopropanol (100%)	2 ml	8 ml
Total volume	**4 ml**	**16 ml**

2. Briefly spin the tubes, place into the DiaMag02 or DiaMag1.5, wait 1 minute and discard the buffer. Keep the captured beads and add per tube, 100 µl Wash buffer 1. Close the tubes, invert the 8-tube strip to resuspend the beads and incubate for 5 minutes at room temperature on a rotating wheel (40 rpm).
3. Prepare the Wash buffer 2 containing 50% isopropanol as follows:

Wash buffer 2	24 rxns
Wash buffer 2 w/o isopropanol	2 ml
Isopropanol (100%)	2 ml
Total volume	**4 ml**

4. Briefly spin the tubes, place into the DiaMag02 or DiaMag1.5, wait 1 minute and discard the buffer. Keep the captured beads and add 100 µl Wash buffer 2 per tube. Close the tubes, invert the 8-tube strip to resuspend the beads and incubate for 5 minutes at room temperature on a rotating wheel (40 rpm).

STEP 4: DNA elution (ChIP & MeDIP)

Two elutions in 25 µl (total volume 50 µl; option I)

Note: If required to finish up at a precise concentration in a specific buffer we suggest option 2 (see page 14).

1. Briefly spin the tubes and place them into the DiaMag02 or DiaMag1.5, wait 1 minute and discard the buffer. Keep the captured beads and add per tube, 25 µl buffer C. Close the tubes, invert the 8-tube strip to resuspend the beads and incubate for 15 minutes at room temperature on a rotating wheel (40 rpm). Resuspend the pelleted beads using the pipet and make sure that you drop them on the bottom of the tube.
2. Spin the two 8-tube strips and place it into the DiaMag02 or DiaMag1.5, wait 1 minute and transfer the supernatants into a new labelled 1.5 ml tube. Keep the bead pellets on ice.
3. Repeat the elution of the bead pellets for 15 min at room temperature on a rotating wheel (40 rpm) in 25 µl buffer C.
4. Spin the two 8-tube strips and place it in the DiaMag02 or DiaMag1.5, wait 1 minute and pool the supernatant with the corresponding IP or input sample (1.5 ml tube). Discard the beads.
5. Place the DNA on ice and proceed to any desired downstream applications, or store it at -20°C or -80°C until further use.

Detailed protocol

After the last wash of immunoprecipitated material (ChIP or MeDIP), discard the last traces of wash buffer and use the pellet of beads (8-tube strip) for STEP 1a (ChIP) or STEP 1b (MeDIP).

STEP 1a. Chromatin reverse crosslinking and elution (ChIP)

1. Prepare the **Elution buffer** by mixing **Buffer A** and **B** as follows:

Elution buffer	1 rxn
Buffer A	115.4 µl
Buffer B	4.6 µl
Total volume	**120 µl**

- Place Buffer A at 25°C during 30 min before use.
- 100 µl of Elution buffer are needed per IPure reaction (20 µl excess).
- 1 IPure reaction corresponds to the purification 1 ChIP or 1 input sample.

<u>Attention:</u>
Make sure when working with Buffer A, that there are no crystals left in solution. Otherwise heat up gently and mix until complete disappearence of such crystals.

2. Add **100 µl of Elution buffer** to the bead pellets (tube strip).

3. Thaw your input sample (1.5 ml tube), and perform a pulse spin. Add **90 µl of Elution buffer** and **10 µl of input sample** to a new 200 µl tube (8 tube-strip).
   - Input sample corresponds to 10% of the IP sample.

4. Incubate samples and input for 4 hours (or overnight) at 65°C on a thermomixer, with continuous shaking.

5. Spin the 8-tube strip and place it into the **DiaMag02 or DiaMag1.5** (magnetic rack). After 1 minute, transfer the supernatants to a new labelled 8-tube strip. Keep the samples on ice.

STEP 1b. DNA elution (MeDIP)

1. Prepare the **Elution buffer** by mixing **Buffer A** and **B** as follows:

Elution buffer	1 rxn
Buffer A	115.4 µl
Buffer B	4.6 µl
Total volume	**120 µl**

- Place Buffer A at 25°C during 30 min. before use.
- 100 µl of Elution buffer are needed per IPure reaction (20 µl excess).
- 1 IPure reaction corresponds to the purification 1 MeDIP or 1 input sample.

Attention:
Make sure when working with Buffer A, that there are no crystals left in solution. Otherwise heat up gently and mix until complete disappearence of such crystals.

2. Add **50 µl of Elution buffer** to the bead pellets (tube strip).

3. Thaw your input sample (1.5 ml tube), and perform a pulse spin. Add **92.5 µl of Elution buffer** and **7.5 µl of input sample** to a new 200 µl tube (8-tube strip).
   - Input sample corresponds to 10% of the IP sample.

4. Incubate samples and input DNA for **15 min at room temperature** on a rotating wheel (40 rpm).

5. Spin the 8-tube strip and place it into the **DiaMag02 or DiaMag1.5** (magnetic rack). After 1 minute, transfer the supernatants to a new labelled 8-tube strip.

6. Repeat the incubation of the bead pellets for **15 min at room temperature** on a rotating wheel (40 rpm) in **50 µl elution buffer**.
   - For input DNA samples: 1 elution in 100 µl.
   - For MeDIP samples: 2 elutions in 50 µl (total volume 100 µl).

7. Spin the 8-tube strip. Place the 8-tube strips into the **DiaMag02 or DiaMag1.5** containing the 50 µl IP samples, wait 1 minute and transfer the supernatants to the new labelled 8-tube strip to pool with the corresponding IP samples.
   - Elutions of IP and input samples are now completed in 100 µl and are in the same 8-tube strip.

STEP 2. DNA binding (ChIP & MeDIP)

1. **Add 2 µl of carrier** to each IP and input sample. Vortex briefly and perform a short spin.

2. Add **100 µl of 100% isopropanol** to each IP and input sample. Vortex briefly and perform a short spin.

Following the addition of isopropanol the solution may become cloudy. This is not detrimental to your experiment and will not influence the quality or quantity of your purified DNA.

3. Resuspend the provided **Magnetic beads** and transfer 15 µl to each IP and input sample.
   - Keep Magnetic beads in liquid suspension during storage at 4°C and at all handling steps, as drying will result in reduced performance.
   - The final volume is now 217 µl per IPure reaction.

4. Incubate IP and input samples for 1 hour at room temperature on a rotating wheel (40 rpm).

STEP 3. Washes (ChIP & MeDIP)

1. Prepare the **Wash buffer 1** containing 50% isopropanol for 24 reactions:

Wash buffer 1	24 rxns
Wash buffer 1 w/o isopropanol	2 ml
Isopropanol (100%)	2 ml
Total volume	**4 ml**

- Never leave the bottle open to avoid evaporation.

2. Briefly spin the tubes, place in the **DiaMag02 or DiaMag1.5**, wait 1 minute and discard the buffer. Keep the captured beads and add per tube, **100 µl Wash buffer 1**. Close the tubes, invert the 8-tube strip to resuspend the beads and incubate for 5 minutes at room temperature on a rotating wheel (40 rpm).
   - Do not disturb the captured beads attached to the tube wall.
   - Always briefly spin the tubes to bring down liquid caught in the lid prior to positioning into the Diagenode Magnetic Rack.

3. Prepare the **Wash buffer 2** containing 50% isopropanol as follows:

Wash buffer 2	24 rxns
Wash buffer 2 w/o isopropanol	2 ml
Isopropanol (100%)	2 ml
Total volume	**4 ml**

   - Never leave the bottle open to avoid evaporation.

4. Wash the IP and input samples with the **Wash buffer 2** as follows. Briefly spin the tubes, place into the **DiaMag02 or DiaMag1.5**, wait 1 minute and discard the buffer. Keep the captured beads and add per tube, **100 µl Wash buffer 2**. Close the tubes, invert the 8-tube strip to resuspend the beads and incubate for 5 minutes at room temperature on a rotating wheel (40 rpm).
   - Do not disturb the captured beads attached to the tube wall.
   - Always briefly spin the tubes to bring down liquid caught in the lid prior to positioning in the **DiaMag02**.

STEP 4. DNA elution (ChIP & MeDIP)

Note: This elution buffer (Buffer C) is suitable for direct qPCR analysis, whole genome amplification, chip hybridization and next generation sequencing.

We propose two options for this step. **Option 1** consists of two elutions in 25 µl each (standard procedure). However if you need to finish up at a precise concentration and in a specific buffer, we suggest to use **option 2**. This option allows for precipitation of the DNA and resuspension of the dried pellet in the volume of choice (e.g. 20 µl of water, see below). For some downstream applications specific concentrations and buffers might be required.

a) Option 1: Two elutions in 25 µl (total volume: 50 µl)

1. Briefly spin the tubes and place them into the **DiaMag02 or DiaMag1.5**, wait 1 minute and discard the buffer. Keep the captured beads and add per tube, **25 µl buffer C**. Close the tubes, invert the 8-tube strip to resuspend the beads and incubate for **15 minutes at room temperature** on a rotating wheel (40 rpm). Resuspend the pelleted beads using the pipet and make sure that you drop them on the bottom of the tube.

2. Spin the two 8-tube strips and place it into the **DiaMag02 or DiaMag1.5**, wait 1 minute and transfer the supernatants into a new labelled 1.5 ml tube. Keep the bead pellets on ice.

3. Repeat the elution of the bead pellets for **15 min at room temperature** on a rotating wheel (40 rpm) in **25 µl buffer C**.

4. Spin the two 8-tube strips and place it into the **DiaMag02 or DiaMag1.5**, wait 1 minute and pool the supernatant with the corresponding IP or input sample (1.5 ml tube). Discard the beads.
   - Total elution volume for both IP and input samples is 50 µl (1.5 ml tube).

5. Place the DNA on ice and proceed to any desired downstream applications, or store it at -20°C or -80°C until further use.

b) Option 2: Two elutions in 75 µl followed by ethanol precipitation

1. Briefly spin the tubes, place into the **DiaMag02 or DiaMag1.5**, wait 1 minute and discard the buffer. Keep the captured beads and add per tube, **75 µl buffer C**. Close the tubes, invert the 8-tube strip to resuspend the beads and incubate for **15 minutes at room temperature** on a rotating wheel (40 rpm).

2. Spin the two 8-tube strips and place it into the **DiaMag02 or DiaMag1.5**, wait 1 minute and transfer the supernatants to new labelled 1.5 ml tubes. Keep the bead pellets on ice.

3. Repeat the elution of the bead pellets for **15 min at room temperature** on a rotating wheel (40 rpm) in **75 µl of buffer C**.

4. Spin the two 8-tube strips and place it into the **DiaMag02 or DiaMag1.5**, wait 1 minute and pool the supernatants with the corresponding IP or input sample (1.5 ml tube). Discard the beads.
   - Total elution volume for both IP and input samples is 150 µl (1.5 ml tube).

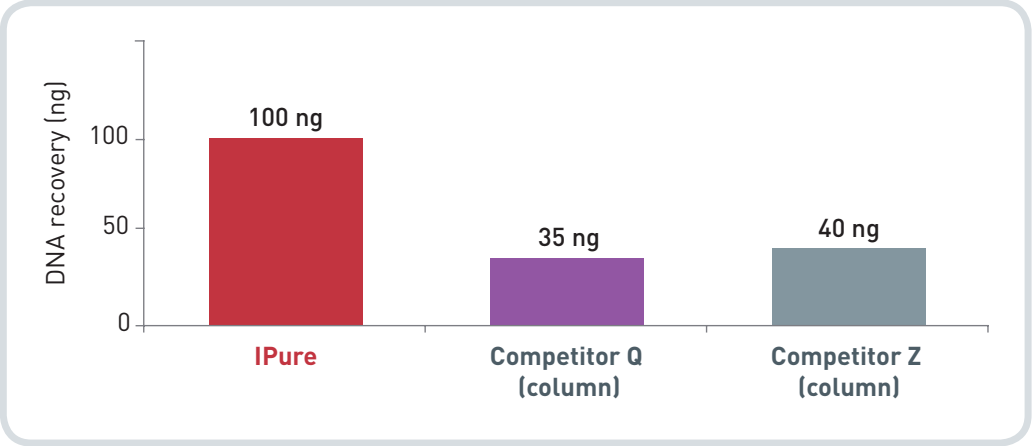
Ethanol Precipitation

Attention: reagents not provided for ethanol precipitation!

5. Put all the tubes on ice.
6. Add per tube **1 µl of Glycogen, 15 µl of Sodium Acetate** and **300 µl of cold 100% ethanol**.
7. Mix well and incubate at -20°C for at least 2 hours.
8. Centrifuge the tube for 15 minutes, 14000 rpm at 4°C.
9. Carefully discard the supernatant without disturbing the pellet.
10. Add 500 µl of cold **70% ethanol**
11. Centrifuge the tube for 5 minutes, 14000 rpm at 4°C.
12. Carefully discard the supernatant without disturbing the pellet.
13. Air-dry the pellet for ~5 minutes (do not completely dry the pellet).
14. Resuspend the DNA in **20 µl** (or any other volume) **of DNase-free water or other buffer**.
15. Place the DNA on ice and proceed to any desired downstream applications, or store the DNA at -20°C or below until further use.

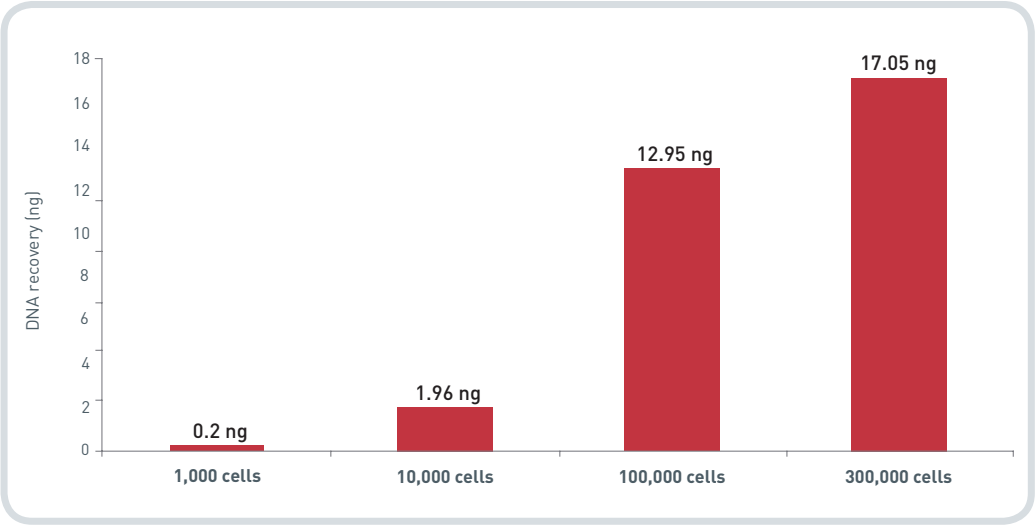
Results

Comparison of DNA recovery after purification with IPure technology and competitor kits



MeDIP assays were performed using the MagMeDIP kit (cat# mc-magme-048). The immunoprecipitated samples were purified with the IPure technology and two competitor kits (competitor Q and Z). The purified DNA was eluted in 50 µl of water and quantified with a Nanodrop.

DNA recovery after purification of ChIP samples using IPure technology



ChIP assays were perfomed using different amounts of U2OS cells, the LowCell# ChIP kit (cat# kch-maglow-016) and the H3K9me3 antibody (Cat. No. pAb-056-050; 2 µg/IP). The purified DNA was eluted in 50 µl of water and quantified with a Nanodrop.

Ordering information

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MANUAL

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

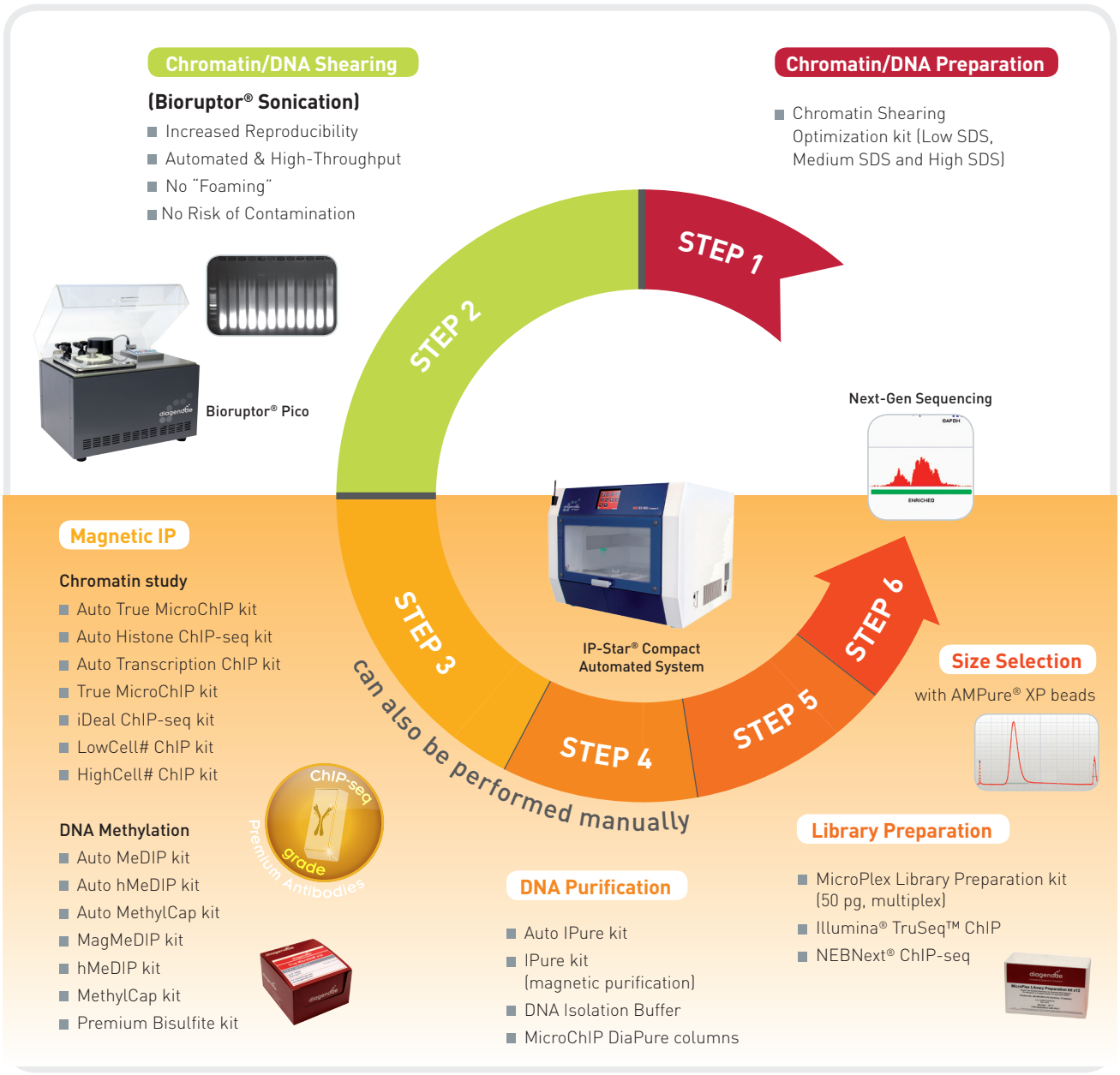

Figure 1. Diagenode provides a full suite of manual and automated solutions for ChIP experiments.

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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