diagenode

A Hologic Company

iPure kit v2

Magnetic DNA Purification kit for epigenetic applications

Cat. No. C03010014 (x24)

Cat. No. C03010015 (x100)

USER GUIDE

V5 08_2023

Please read this manual carefully
before starting your experiment

Contents

Introduction

Diagenode's **IPure kit** is the only DNA purification kit using magnetic beads, that is specifically optimized for extracting DNA from **ChIP**, **MeDIP** and **CUT&Tag** (Chromatin Immunoprecipitation, Methylated DNA Immunoprecipitation and Cleavage Under Targets & Tagmentation).

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.

The kit IPure can be used with the Diagenode's DiaMag02 magnetic rack:

DiaMag02 - magnetic rack
Cat. No. B04000001

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

Kit Method Overview

IPure after ChIP		IPure after MeDIP		IPure after CUT&Tag	
 **STEP 1A.** Chromatin reverse cross-linking and elution	4 hours or overnight	 **STEP 1B.** DNA elution	30 min	 **STEP 1C.** pA-Tn5 inactivation and DNA release	30 min
 **STEP 2A.** DNA binding	10 min	 **STEP 2B.** DNA binding	10 min	 **STEP 2C.** DNA binding	10 min
 **STEP 3.** Washes	1 min	 **STEP 3.** Washes	1 min	 **STEP 3.** Washes	1 min
 **STEP 4.** DNA elution	15 min	 **STEP 4.** DNA elution	15 min	 **STEP 4.** DNA elution	15 min
TOTAL TIME	4 hours 30 min		1 hour		1 hour

Workflow description

IPure after ChIP

Step 1: Chromatin is decrosslinked and eluted from beads (magnetic or agarose) which are discarded. Magnetic beads for purification are added.

Step 2: Magnetic beads acquire positive charge to bind the negatively charged phosphate backbone of DNA. DNA-bead complex is separated using a magnet.

Step 3: Proteins and remaining buffer are washed away.

Step 4: DNA is eluted from magnetic beads, which are discarded. Purified DNA is ready for any downstream application (NGS, qPCR, amplification, microarray).

IPure after MeDIP

Step 1: DNA is eluted from beads (magnetic or agarose) which are discarded. Magnetic beads for purification are added.

Step 2: Magnetic beads acquire positive charge to bind the negatively charged phosphate backbone of DNA. DNA-bead complex is separated using a magnet.

Step 3: Remaining buffer are washed away.

Step 4: DNA is eluted from magnetic beads, which are discarded. Purified DNA is ready for any downstream application (NGS, qPCR, amplification, microarray).

IPure after CUT&Tag

Step 1: pA-Tn5 is inactivated and DNA released from the cells. Magnetic beads for purification are added.

Step 2: Magnetic beads acquire positive charge to bind the negatively charged phosphate backbone of DNA. DNA-bead complex is separated using a magnet.

Step 3: Proteins and remaining buffer are washed away.

Step 4: DNA is eluted from magnetic beads, which are discarded. Purified DNA is ready for any downstream application (NGS, qPCR, amplification, microarray).

Kit Content

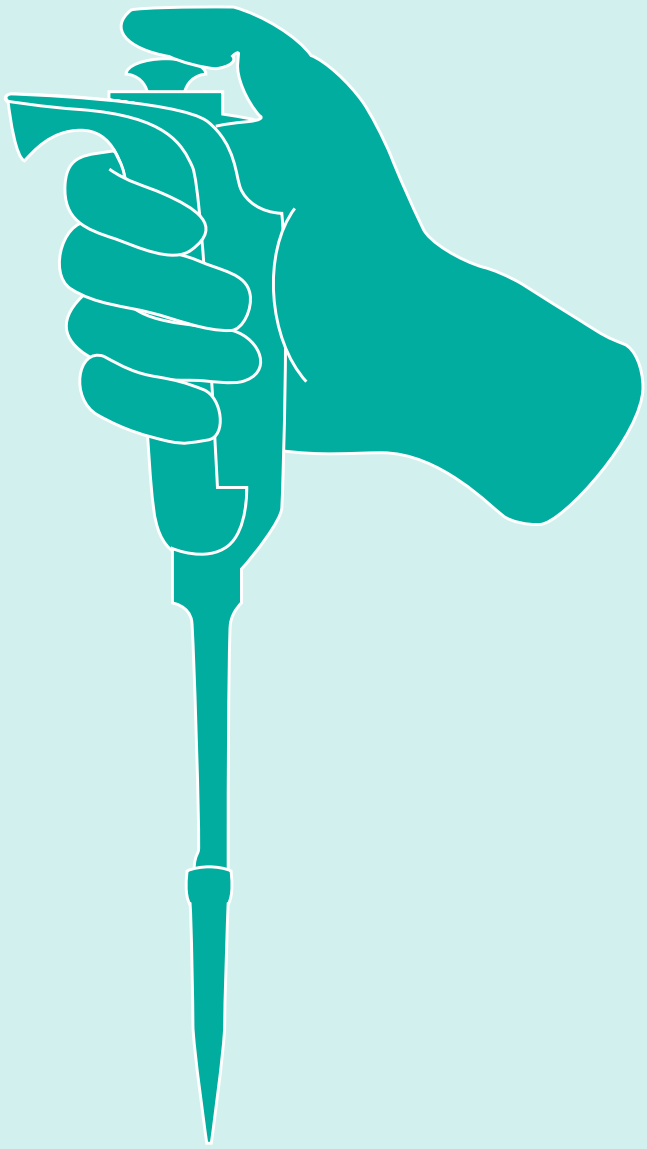
The kit content is sufficient to perform 24 (C03010014) or 100 reactions (C03010015).

IPure Kit			
Description	**Quantity (x24)**	**Quantity (x100)**	**Storage**
200 µl tube strips (8 tubes/strip) + cap strips	4 pc	15 pc	Room temperature
Buffer A	2.8 ml	10 ml	4°C
Buffer B	115 µl	460 µl	4°C
Wash buffer 1 w/o isopropanol	1.5 ml	9 ml	4°C
Wash buffer 2 w/o isopropanol	1.5 ml	9 ml	4°C
Buffer C	1.2 ml	9 ml	4°C
IPure Beads v3	240 µl	1 ml	4°C
Carrier*	48 µl	200 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).*

Required Materials Not Provided

- DiaMag02 - magnetic rack (B04000001)
- 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
- For CUT&Tag:
    - 0.5M EDTA
    - 10% SDS
    - Thermolabile proteinase K (eg New England Biolabs P8111S)



PROTOCOL

STEP 1A

Chromatin Reverse Crosslinking and Elution

After the last wash of immunoprecipitated material, discard the last traces of wash buffer and use the pellet of beads (8-tube strip) for STEP 1.1.

1.1 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 minutes before use.
- 100 µl of Elution Buffer are needed per IPure reaction (20 µl excess).
- 1 IPure reaction corresponds to the purification of 1 ChIP or 1 input sample.

NOTE: *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.*

1.2 Add **100 µl of Elution Buffer** to the bead pellets (tube strip).

1.3 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).

NOTE: *Input sample corresponds to 10% of the IP sample.*

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

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

STEP 2A

DNA Binding

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

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

NOTE: *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.*

2.3 Resuspend the provided **Magnetic beads** and transfer 10 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 212 µl per IPure reaction.

2.4 Incubate IP and input samples for 10 minutes at room temperature on a rotating wheel (40 rpm).

STEP 1B

DNA Elution

After the last wash of immunoprecipitated material, discard the last traces of wash buffer and use the pellet of beads (8-tube strip) for STEP 1.1.

1.1 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 minutes before use.
- 100 µl of Elution Buffer are needed per IPure reaction (20 µl excess).
- 1 IPure reaction corresponds to the purification of 1 MeDIP or 1 input sample.

NOTE: *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.*

1.2 Add **50 µl of Elution Buffer** to the bead pellets (tube strip).

1.3 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).

NOTE: *Input sample corresponds to 10% of the IP sample.*

1.4 Incubate samples and input DNA for 15 minutes at room temperature on a rotating wheel (40 rpm).

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

1.6 Repeat the incubation of the bead pellets for 15 minutes 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).

1.7 Spin the 8-tube strip. Place the 8-tube strips into the **DiaMag02** 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 2B

DNA Binding

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

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

NOTE: *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.*

2.7 Resuspend the provided **Magnetic beads** and transfer 10 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 212 µl per IPure reaction.

2.8 Incubate IP and input samples for 10 minutes at room temperature on a rotating wheel (40 rpm).

STEP 1C

pA-Tn5 Inactivation and DNA Release

***NOTE**: the protocol below is compatible with CUT&TAG version described by Kaya-Okur, H.S., Wu, S.J., Codomo, C.A. et al. CUT&TAG for efficient epigenomic profiling of small samples and single cells. Nat Commun 10, 1930 (2019). https://doi.org/10.1038/s41467-019-09982-5*

1.1 After completion of the tagmentation reaction, add **10 µl of 0.5 M EDTA**, **3 µl of 10% SDS** and **1 µl of thermolabile proteinase K** directly to each sample and mix by full-speed vortexing for 2 seconds. Do not remove the Tagmentation Buffer and Concavalin A beads.

1.2 Incubate at 37ºC for 15 minutes.

1.3 Heat samples at 55ºC for 10 minutes to inactivate thermolabile proteinase K.

STEP 2C

DNA Binding

***NOTE**: an equival volume of isopropanol should be added. If the tagmentation reaction volume is different from 300 µl, adjust the required volume of isopropanol.*

2.1 Add 300 µl of 100% isopropanol to each sample. Vortex briefly and perform a short spin. Do not remove Concavalin A beads. 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.

***NOTE**: an equival volume of isopropanol should be added. If the tagmentation reaction volume differs from 300 µl, adjust the required volume of isopropanol.*

2.2 Re-suspend the provided Magnetic beads and transfer 10 µl to each 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 624 µl per IPure reaction.

2.3 Incubate samples for 10 minutes at room temperature on a rotating wheel (40 rpm).

STEP 3

Washes (ChIP, MeDIP and CUT&TAG)

3.1 Prepare the **Wash Buffer 1** containing 50% isopropanol for 100 reactions:

Wash Buffer 1	24 rxns	100 rxns
Wash Buffer 1 w/o Isopropanol	1.5 ml	9 ml
Isopropanol (100%)	1.5 ml	9 ml
Total Volume	3 ml	18 ml

- Never leave the bottle open to avoid evaporation.

3.2 Briefly spin the tubes, place in the **DiaMag02**, 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.

3.3 Prepare the **Wash Buffer 2** containing 50% isopropanol as follows:

Wash Buffer 2	24 rxns	100 rxns
Wash Buffer 2 w/o Isopropanol	1.5 ml	9 ml
Isopropanol (100%)	1.5 ml	9 ml
Total Volume	3 ml	18 ml

- Never leave the bottle open to avoid evaporation.

3.4 Wash the IP and input samples with the **Wash Buffer 2** as follows. Briefly spin the tubes, place into the **DiaMag02**, 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 and CUT&TAG)

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

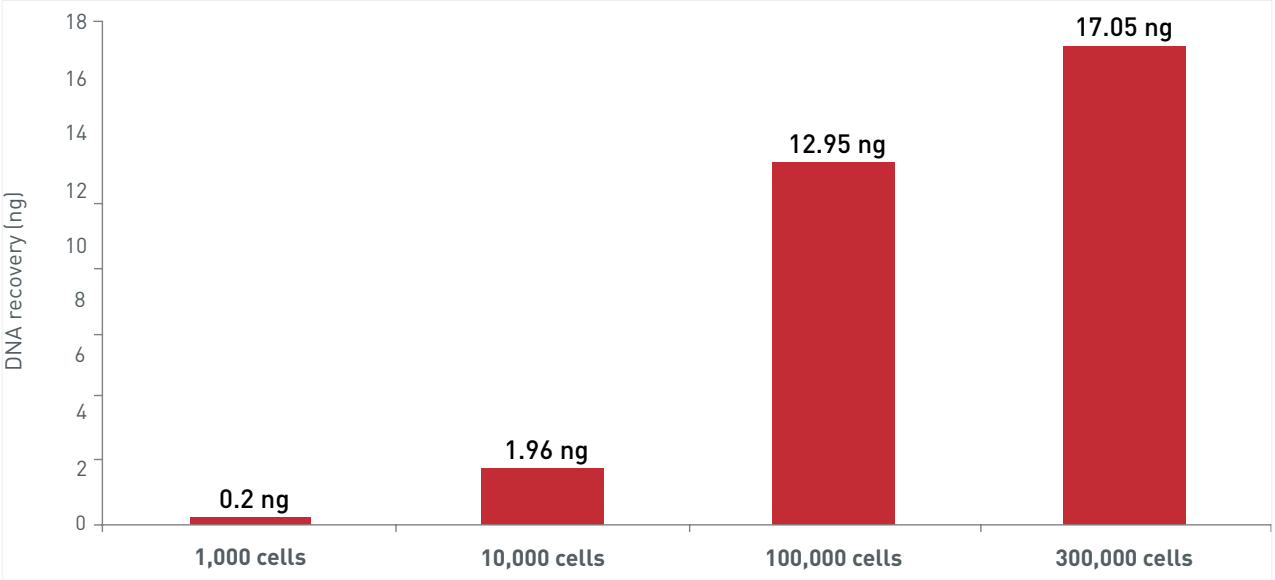
4.1 Briefly spin the tubes and place them into the **DiaMag02**, wait 1 minute and discard the buffer. Keep the captured beads and add per tube, **25 µl of Buffer C** (alternatively, a higher volume can be used for the elution if necessary). Close the tubes, invert the 8-tube strip to resuspend the beads 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.

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

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

Results

DNA recovery after purification of ChIP samples using IPure technology



ChIP assays were perfomed using different amounts of U2OS cells, the LowCell# ChIP kit and the H3K9me3 antibody (Cat. No. C15410056; 2 µg/IP). The purified DNA was eluted in 50 µl of water and quantified with a Nanodrop.

Related Products

Description	Cat. No.	Format
DiaMag02 - magnetic rack	B04000001	1 pc
200 µl tube strips (8 tubes/strip) + cap strips	C30020002	120 pc
iDeal ChIP-seq kit for Histones x10	C01010050	10 rxns
iDeal ChIP-seq kit for Transcription Factors x10	C01010054	10 rxns
MagMeDIP kit x10	C02010020	10 rxns
MagMeDIP kit x48	C02010021	48 rxns
pA-Tn5 loaded	C01070001	15 µl/30 µl
pA-Tn5 unloaded	C01070002	10 µl/30 µl

Revision history

Version	Date of modification	Description of modifications
V5 08_2023	August 2023	Correction of small error page 17, step 2.2.
V5 02_2023	February 2023	- Replacement of the IPure beads v2 by IPure beads v3 - Removal of obsolete reference (DiaMag1.5)

FOR RESEARCH USE ONLY.

Not intended for any animal or human therapeutic or diagnostic use.

NOTICE TO PURCHASERLIMITED LICENSE

The information provided herein is owned by Diagenode and/or its affiliates. Subject to the terms and conditions that govern your use of such products and information, Diagenode and/or its affiliates grant you a nonexclusive, nontransferable, non-sublicensable license to use such products and information only in accordance with the manuals and written instructions provided by Diagenode and/or its affiliates. You understand and agree that except as expressly set forth in the terms and conditions governing your use of such products, that no right or license to any patent or other intellectual property owned or licensable by Diagenode and/or its affiliates is conveyed or implied by providing these products. In particular, no right or license is conveyed or implied to use these products in combination with any product not provided or licensed to you by Diagenode and/or its affiliates for such use. Limited Use Label License: Research Use Only The purchase of this product conveys to the purchaser the limited, non-transferable right to use the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee orother form of consideration. For information on obtaining additional rights, please contact info@diagenode.com.

TRADEMARKS

The trademarks mentioned herein are the property of Diagenode or their respective owners. Bioanalyzer is a trademark of Agilent Technologies, Inc. Agencourt and AMPure® are registered trademarks of Beckman Coulter, Inc. Illumina® is a registered trademark of Illumina®Inc; Qubit is a registered trademark of Life Technologies Corporation.

www.diagenode.com