diagenode

A Hologic Company

Auto IPure kit V2

Magnetic DNA Purification kit for epigenetic applications

Cat. No. **C03010010** (100 rxns)

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For a complete listing of Diagenode's international distributors visit:
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For rest of the world, please contact Diagenode sa.

Diagenode website: www.diagenode.com

Content

Introduction

The Diagenode IP-Star® Automated System automates immunoprecipitation and increases reproducibility

Diagenode, the leading provider of complete solutions for epigenetics research, offers a variety of end-to-end systems to streamline DNA methylation and chromatin immunoprecipitation workflows. Central to this full offering is Diagenode's Automated Systems, simple yet robust automated bench-top instruments that standardize different epigenetic applications (i.e. ChIP, MeDIP or MethylCap). Diagenode designed these automation systems to make ChIP and DNA methylation studies accessible and reproducible, and ensure consistent data in every experiment.

Diagenode Automated Systems will produce consistent results from any operator regardless of the day, the experimental run, or the lab. Robust and reproducible results is a major goal of today's high resolution epigenomic studies.

Diagenode Automated Platforms replace the numerous manual, error-prone steps of complex epigenetic applications with a reliable, highly consistent and automated process that requires minimal operator intervention. We empower researchers to simplify the tedious protocols and the complexity of many epigenetic protocols. In addition, Diagenode Automated Systems minimize sample carryover, data variability, and costly errors. The platforms offer full workflow support for epigenetics research, utilizing our complete kits and laboratory-validated protocols to rapidly deliver high-quality and consistent data.

Auto IPure kit V2

Diagenode's Auto IPure kit v2 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) experiments.

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 page...). 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.

Diagenode's IP-Star system uses the principle of bead-based magnetic separation. Magnetic beads bound with chromatin or DNA are brought to the inner wall of the tip when a strong magnetic force is applied. This differs from other systems that collect the bound DNA on the bottom of a reaction well, resulting in cleaner assays and less carryover.

IP-Star® and IP-Star® Compact Systems for automation of epigenetic applications

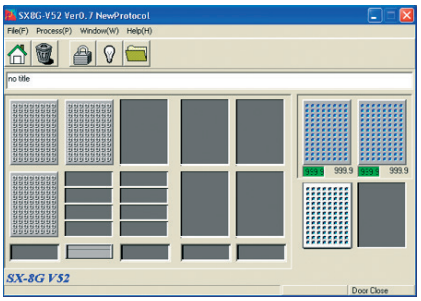
Diagenode has developed two automated platforms (IP-Star® and IP-Star® Compact) designed to increase your lab's productivity, efficiency and experimental reproducibility. The two automated platforms are capable of processing up to 16 samples per cycle. The automated systems processes sheared chromatin (or DNA) to deliver purified DNA ready for qPCR, amplification, microarray and sequencing analysis. Both, the IP-Star® and IP-star® Compact have an easy-to-use open software that provides you with flexibility. This allows you to create your personal protocol according to your specific needs.

Major benefits of Diagenode Automated Platforms

IP-Star® Compact

IP-Star®

- → High resolution ChIP-seq and MeDIP-seq profiles
- → Automated library preparation for Next Generation sequencing
- → Reduces hands on time to just 30 minutes
- → Reduces variability between operators and labs
- → Ideal for low sample starting amounts
- → Compatible with Diagenode Kits
- → Reduces cross-contamination

	IP-Star® Compact	IP-Star®
Applications	ChIP-seq, MeDIP-seq, MethylCap-seq, hMeDIP, IPure, Sample preparation, Re-ChIP, MagBisulfite, RNA-IP, Library preparation for NGS platforms.	ChIP-seq, MeDIP-seq, MethylCap-seq, hMeDIP, IPure, Sample preparation, Re-ChIP, MagBisulfite, RNA-IP.
Software		
User interface	Intuitive touch screen panel	PC Software
User friendly	Software training not required	Software training before use
Dispensing	Automated dispension of assay reagents	Manual dispension of assay reagents
Protocol optimization (flexible parameters)	Antibody coating (temperature, time, mixing speed) Immunoprecipitation (temperature, time, mixing speed) Washes (temperature, time, mixing speed)	Antibody coating (temperature, time) Immunoprecipitation (temperature, time)
New protocol development	Achievable by Diagenode product specialist	Achievable by customer after training
Characteristics	750W x 740 D x 610 H | 100 kg 8 Nozzles X-Y-Z axis | 4 – 95°C	1070W x 650 D x 780 H | 130 kg 8 Nozzles X-Y-Z axis | 4-95°C

Improved reproducibility

Our IP-Star® will increase the immunoprecipitation reproducibility between IPs performed by the same as well as by different operators (see figure 1 and 2 below). Reagents (Antibodies, buffers,...) and sheared chromatin were identical for "ManChIP" and "AutoChIP". The IP-Star® Automated system removes variation that can be created by manual handling and allows you to optimize and standardize your assay within a lab. The IP-Star® is designed to improve the accuracy and the reproducibility of any immunoprecipitiation experiment.

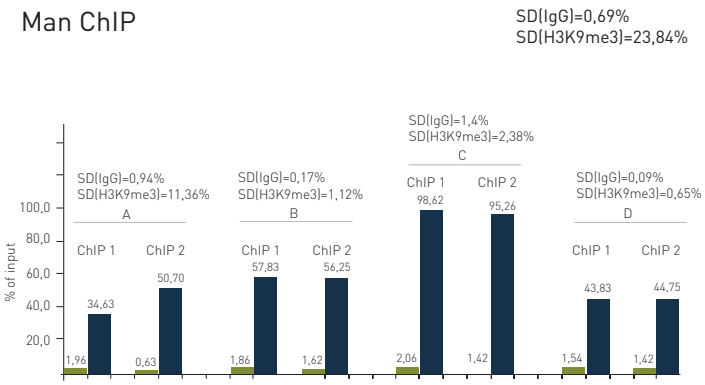


Figure 1. Manual ChIP

Four different operators have each performed two ChIP experiments using H3K9me3 antibody on the genomic region SAT2 (positive locus). 10,000 Hela cells have been used per IP. Reagents and sheared chromatin were identical per assay. The standard deviations between the ChIPs performed by the same operator and between the four different operators are displayed.

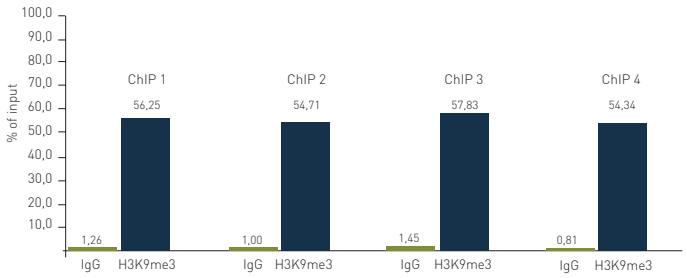
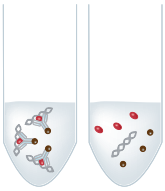


Figure 2. Automated ChIP

Four ChIP experiments using H3K9me3 antibody on the genomic region SAT2 (positive locus) have been performed by the IP-Star®. 10,000 Hela cells have been used per IP. Reagents and sheared chromatin were identical per assay. The standard deviations between the four ChIPs performed by the IP-Star® are displayed.

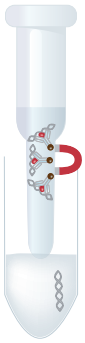
Kit method overview

IPure after ChIP



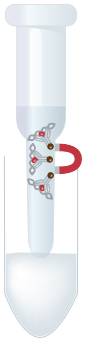
STEP 1.
Chromatin reverse cross-linking and elution

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



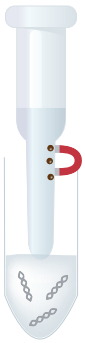
STEP 2. DNA binding

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



STEP 3. Washes

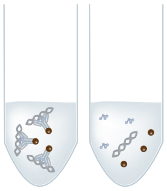
Proteins and antibody debris are washed away.



STEP 4. DNA Elution

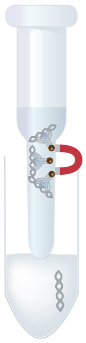
DNA is eluted from magnetic beads, which are discarded.
Purified DNA is ready for any downs-tream application (qPCR, next generation sequencing, amplification, microarray)

IPure after MeDIP



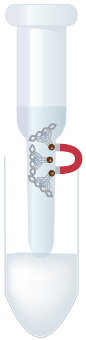
STEP 1.
DNA Elution

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 negatively charged phosphate backbone of DNA.
DNA-bead complex is separated by using a magnet.



STEP 3. Washes

Proteins and antibody debris are washed away.

STEP 4. DNA Elution

DNA is eluted from magnetic beads which are discarded.
Purified DNA is ready for any downs-tream application (qPCR, next generation sequencing, amplification, microarray)

Kit materials

Kit content

The kit content is sufficient to perform 100 reactions.

IPure kit (100 reactions)		
Description	Format	Storage
96 well microplates	10 pc	Room temperature
Buffer A	12 ml	4°C
Buffer B	460 µl	4°C
Wash buffer 1 w/o iso-propanol	9 ml	4°C
Wash buffer 2 w/o iso-propanol	9 ml	4°C
Buffer C	9 ml	4°C
Magnetic beads	1 ml	4°C
Carrier*	200 µl	-20°C

*This product is shipped at 4°C. Store it at -20°C upon arrival.

Plastics and consumables available separately		
Description	Cat. No.	Format
200 µl tube strips (12 tubes/strip) + cap strips	C30020001	80
200 µl tube strips (8 tubes/strip) + cap strips for IP-Star® Compact	C30020002	120
96 well microplates for IP-Star®	C30080030	10
Tips (box)	C30040021	960
Tips (bulk)	C30040020	1000
2 ml microtube for IP-Star® Compact	C30010014	100
Large reagent container for IP-Star® Compact	C30020004	20
Medium reagent container for IP-Star® Compact	C30020003	10

Kits and Modules available separately		
Description	Reference	Quantity
Chromatin shearing optimization kit - Low SDS	C01020010	1 kit
Chromatin shearing optimization kit - Medium SDS	C01020011	1 kit
Chromatin shearing optimization kit - High SDS	C01020012	1 kit
Auto Histone ChIP-seq kit protein A x16	C01010020	16 rxns
Auto Histone ChIP-seq kit protein A x100	C01010022	100 rxns
Auto Histone ChIP-seq kit protein G x16	C01010021	16 rxns
Auto Histone ChIP-seq kit protein G x100	C01010023	100 rxns
Auto MeDIP kit x16	C02010011	16 rxns
Auto MeDIP kit x100	C02010012	100 rxns

IP-STAR® COMPACT

How to perform Automated IPure on the IP-Star® Compact

How to perform Automated IPure on the IP-Star® Compact

Auto IPure is done in 96 well plates placed in the room temperature modules of the IP-Star® Compact.

Each 96 well plate will have capacity to run 8, 16 or 24 IPure samples.

ChIP and MeDIP Elution buffer

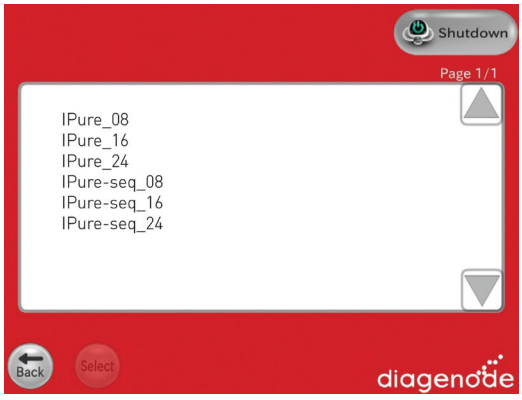
To perform DNA purification with Auto IPure, elution step after Auto Histone ChIP-seq, Auto Plant ChIP-seq and Auto MeDIP kits must be done using the Buffer A and B provided in the Auto IPure kit v2 or Elution Buffer 1 and 2 provided in the ChIP or MeDIP kits.

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

* volume is calculating with 20% of excess

1. Select "**Protocols**" icon and then "**IPure**" category.

2. Select IPure protocol for an elution in 50 µl and IPure-seq protocol for an elution in 25 µl.

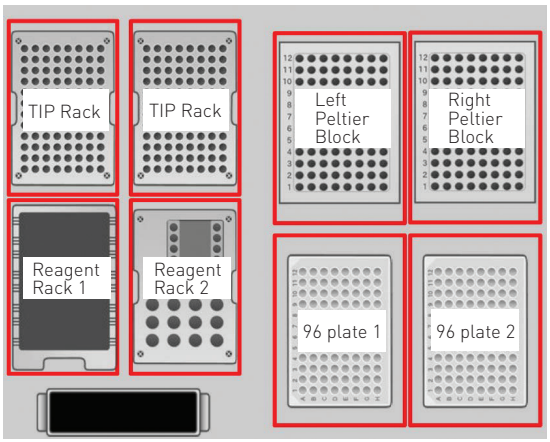


NOTE:
If you plan to run between 1 and 8 samples, choose "**IPure_08** or **IPure-seq_08**"
If you plan to run between 9 and 16 samples, choose "**IPure_16** or **IPure-seq_16**"
If you plan to run between 17 and 24 samples, choose "**IPure_24** or **IPure-seq_24**"

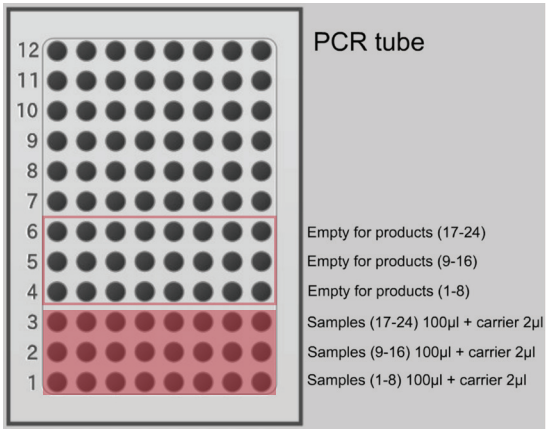
3. Setup the exact number of samples for your experiment. Each IP and input has to be counted as a sample.

   NOTE:
   The Peltier Block is now cooling down to 4°C to keep your samples cold.

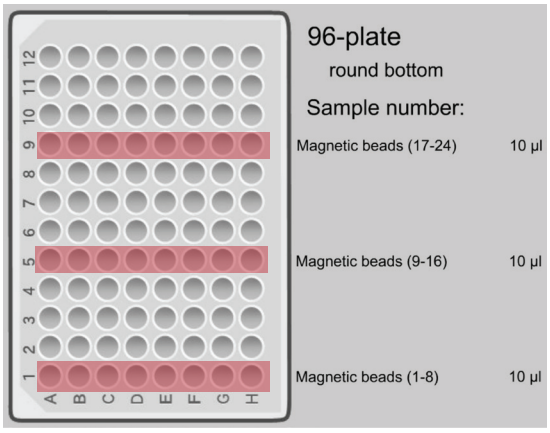
4. Setup all the plastics on the platform according to the screen layout.



5. Add 2 µl of carrier to each IP and input sample and place them on the Left block.



6. Resuspend and dispense 10 µl of magnetic beads (IPure) for each sample on the 96 well plate



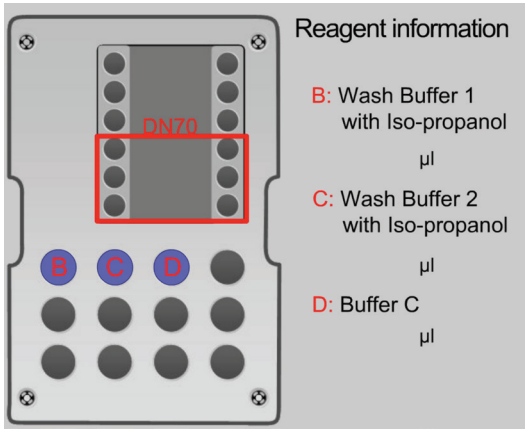
NOTE:
Keep the magnetic beads in liquid suspension during storage at 4°C and at all handling steps, as drying will result in reduced performance. Make sure the beads are homogeneously in suspension at all the time during pipetting steps because the beads are precipitating rapidly.

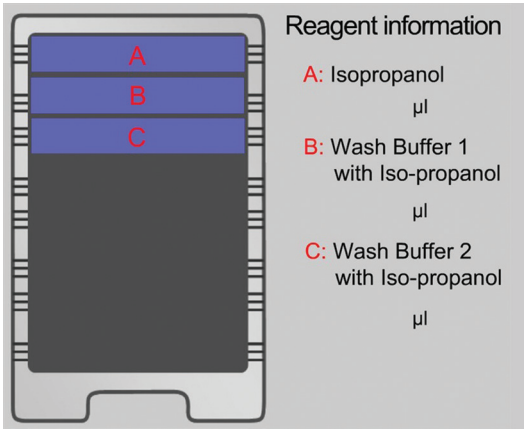
7. Dilute Wash Buffers 1:1 with isopropanol

Wash buffer 1	
Wash buffer 1w/o isopropanol	9 ml
Isopropanol (100%)	9 ml
Total volume	18 ml

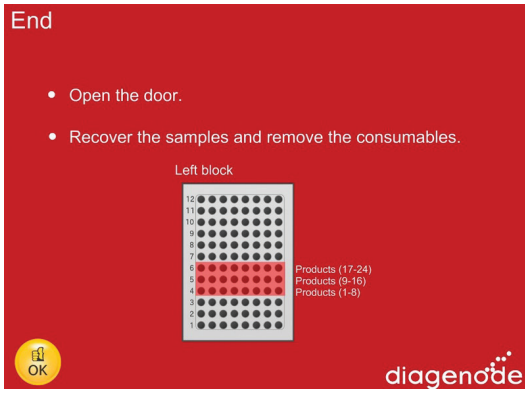
Wash buffer 2	
Wash buffer 1w/o isopropanol	9 ml
Isopropanol (100%)	9 ml
Total volume	18 ml

8. Dispense Wash Buffers 1 & 2 with Isopropanol in the appropriate container in the IP-Star®





9. Dispense Buffer C in the appropriate container in the IP-Star®
10. Press Run to start
11. At the end of the run, recover your samples on the left block at 4°C



12. Press OK, remove the consumables and switch off the IP-Star®
13. Place the DNA on ice and proceed to any desired downstream applications, or store it at -20°C or -80°C until further use.

IP-STAR®

How to perform Automated IPure on the IP-Star®

How to perform Automated IPure on the IP-Star® Compact

Auto IPure is done in 96 well plates placed in the room temperature modules of the IP-Star® Compact.

Each 96 well plate will have capacity to run 8 or16 IPure samples.

A. ChIP and MeDIP Elution buffer

To perform DNA purification with Auto IPure, elution steps after Auto ChIP and Auto MeDIP must be done using the Elution Buffer provided in the Auto IPure kit v2.

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

* volume is calculating with 20% of excess

100 µl Elution Buffer per sample are needed per IP or input sample to perform the elution.

ChIP/MeDIP Elution Buffer provided in the IPure kit will be used as indicated in the Auto ChIP and Auto MeDIP user manuals.

B. Prepare Auto IPure kit buffers

Add, as indicated below, the suggested isopropanol volumes to the corresponding Auto IPure kit v2 buffers.

Wash Buffer 1

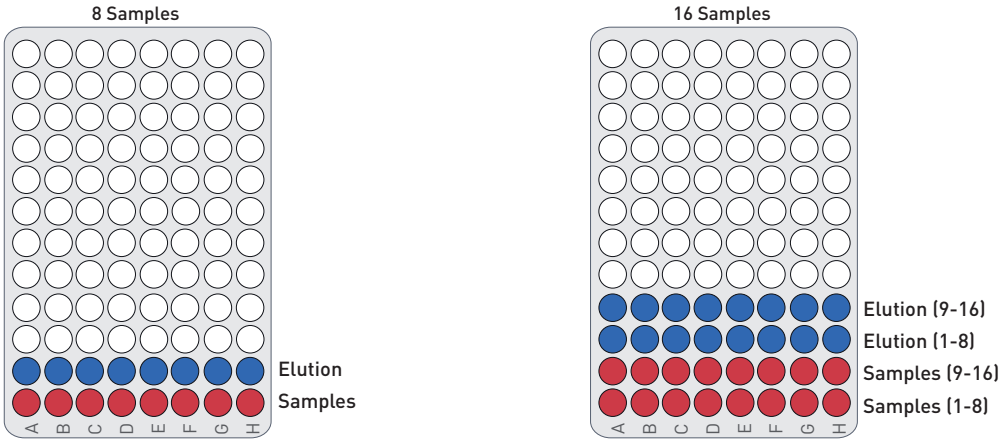
Wash buffer 1	100 rxns
Wash buffer 1 w/o iso-propanol	9 ml
Iso-propanol	9 ml
Total volume	18 ml

Wash Buffer 2

Wash buffer 2	100 rxns
Wash buffer 2 w/o iso-propanol	9 ml
Iso-propanol	9 ml
Total volume	18 ml

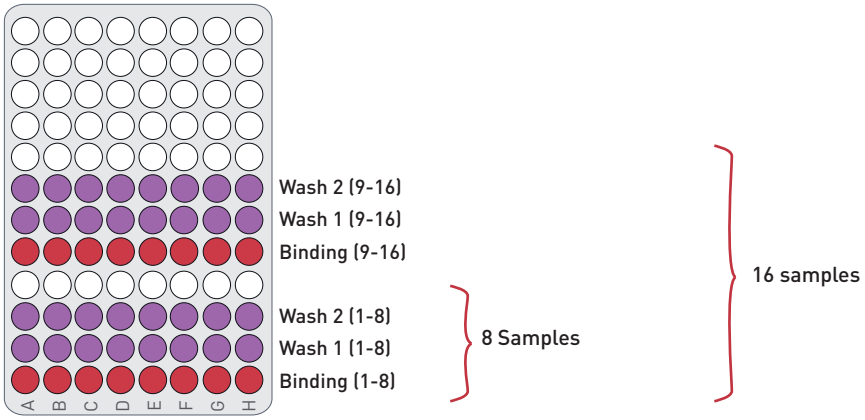
C. Dispense prepared reagents

• Left cooling block



Well	8 samples	16 samples
4		25 µl Buffer C (9-16)
3		25 µl Buffer C (1-8)
2	25 µl Buffer C (1-8)	100 µl sample (9-16) + 100 µl isopropanol + 2 µl carrier
1	100 µl sample (1-8) + 100 µl isopropanol + 2 µl carrier	100 µl sample (1-8) + 100 µl isopropanol + 2 µl carrier

• Left 96 well plate



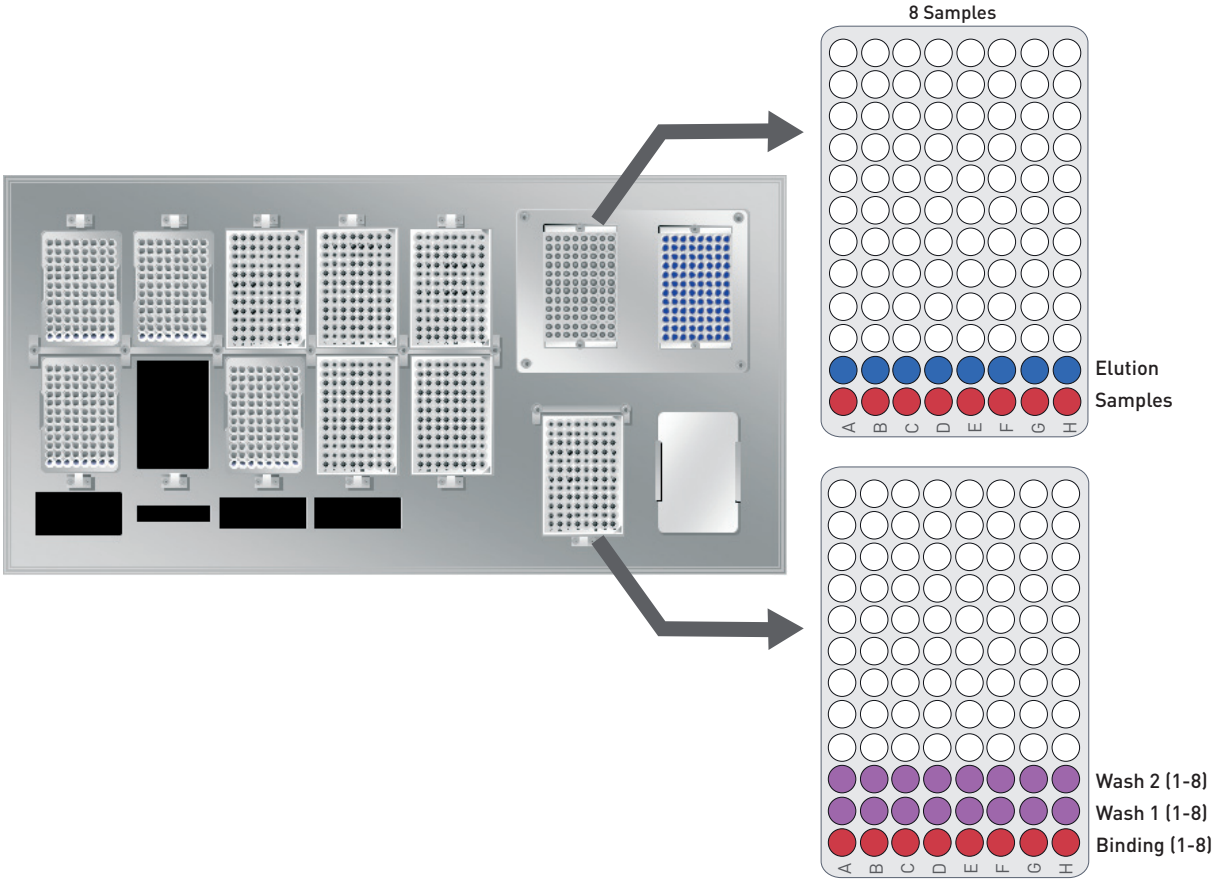
Well	8 samples	16 samples
7		200 µl complete wash buffer 2 (with isopropanol 1:1) (9-16)
6		200 µl complete wash buffer 1 (with isopropanol 1:1) (9-16)
5		10 µl IPure beads v2 (9-16)
4		
3	200 µl complete wash buffer 2 (with isopropanol 1:1) (1-8)	200 µl complete wash buffer 2 (with isopropanol 1:1) (1-8)
2	200 µl complete wash buffer 1 (with isopropanol 1:1) (1-8)	200 µl complete wash buffer 1 (with isopropanol 1:1) (1-8)
1	10 µl IPure beads v2 (1-8)	10 µl IPure beads v2 (1-8)

D. Loading and running protocol

Be sure that the computer connected to the robot never switches to the standby modus (standby modus has to be inactivated). Standby of the computer will lead to the abort of the protocol.

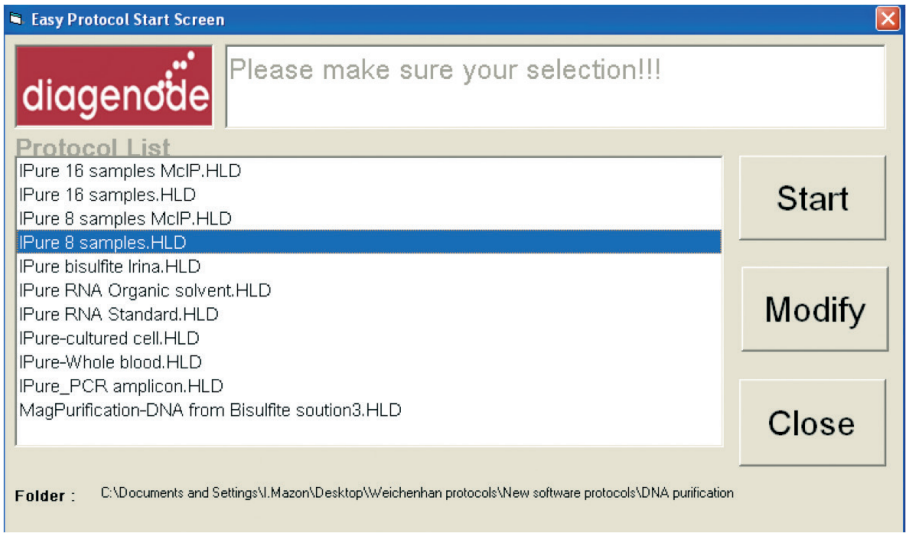
Protocol Name	Auto IPure
Reagent Preparation:	15 min
Binding reaction:	30 min
Washes:	20 min
Elution:	30 min
Total Time:	1:30 min per 8 samples

1. Switch on the SX-8G IP Star. The power switch is on the right side of the instrument.
2. Switch on the computer.
3. Start SX-8G V52 software through SX-8G V52 the following icon
4. Place the prepared 96 well plate in the indicated room temperature module in the workstation

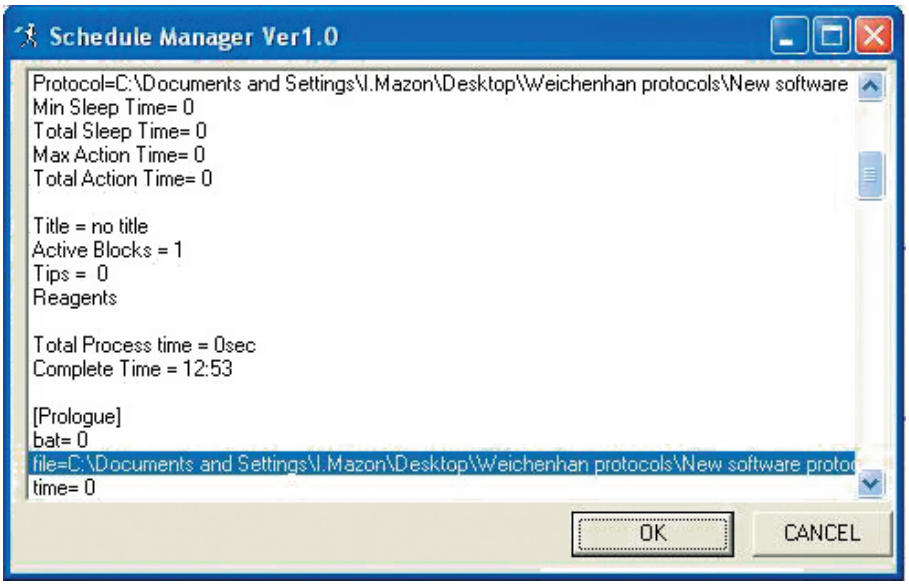


5. Press the following icon 

   Select the protocol of interest. Press start.

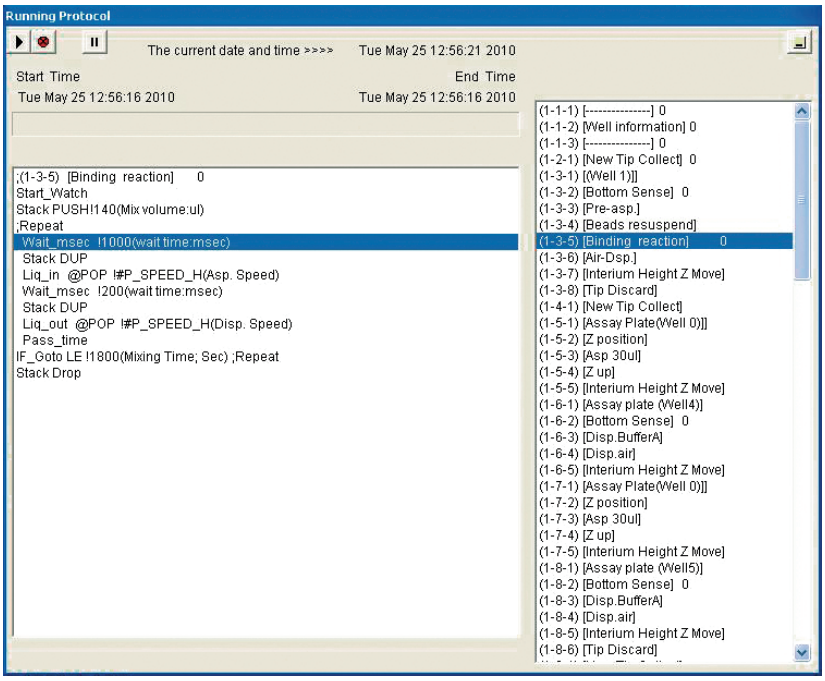


6. Before starting the protocol a start confirmation window will appear. Press OK and the protocol will run.

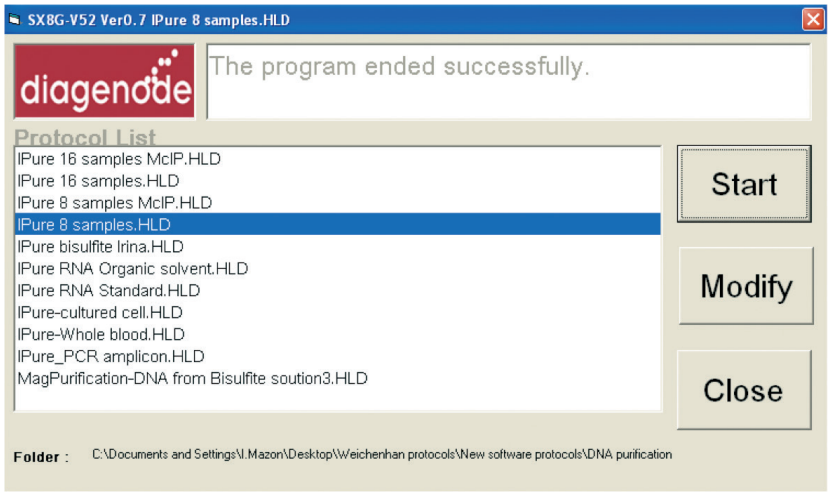


7. The program will run through the following steps: magnetic bead washes, IP and IP washes.

   During protocol the next window will be displayed indicating the current protocol step.



9. The IP-Star software indicates the end of the protocol.
Press the close buttom to finish the protocol run



10. Collect your purified DNA.

This is your DNA ready for qPCR

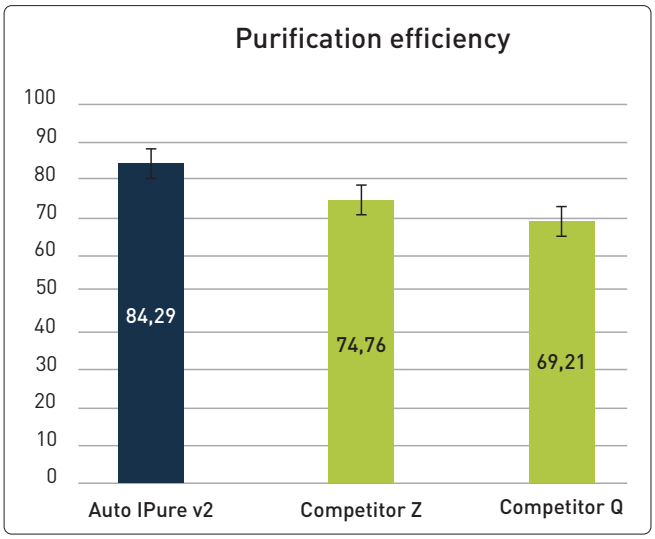
Shutting down the IP-Star

1. Click on File and press End to close the software correctly.
2. Switch off the computer and its monitor.
3. Switch off the IP-Star Robot (power switch on the right side)

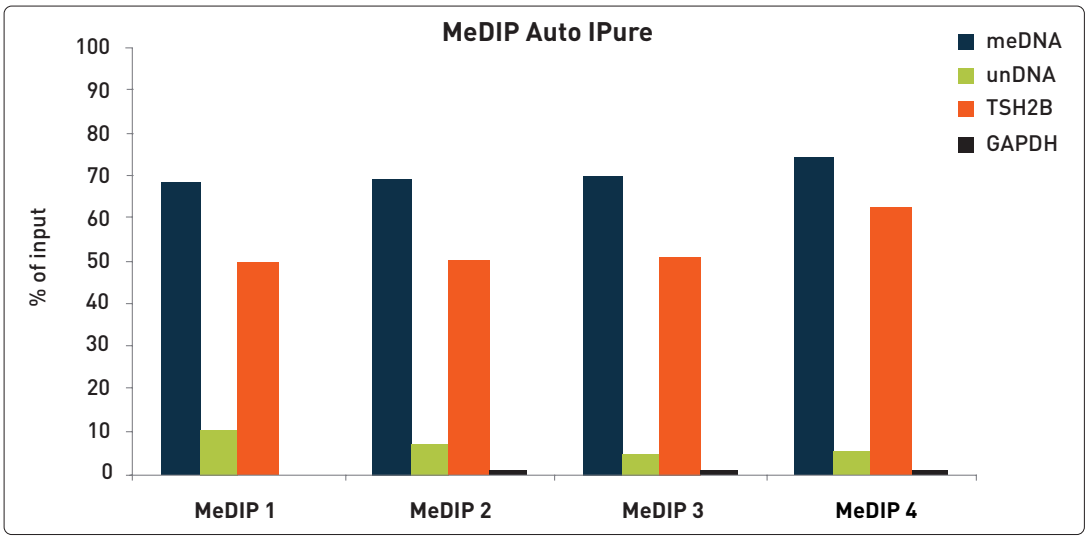
Note: Ensure that the door is closed!

Results

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



DNA recovery after purification of MeDIP samples using IPure technology



Methyl DNA IP results obtained with our Auto MeDIP Kit and after DNA purification using the Auto IPure kit v2

Methyl DNA IP assays were performed using DNA from U2OS cells and the Auto MeDIP kit (Diagenode). After MeDIP, the DNA was purified using the Auto IPure kit v2. Experiments were run in the IP-Star following Diagenode's protocols. The IP was performed by including the kit internal controls: together with the human DNA sample. The internal positive and negative DNA controls included in the IP assay are methylated DNA (meDNA) and unmethylated DNA (unDNA). As positive and negative control regions, a non methylated region in the GAPDH promoter and the methylated region of TSH2B were tested. Results showed 4 different AutoMeDIP-Auto IPure experiments run in the IP-Star automated system.

Troubleshooting guide

Error Cause	Remedy
SX-8G IP-Star cannot be switched on	SX-8G IP-Star is not receiving power. Check that the power cord is connected to the workstation and to the wall power outlet.
Computer cannot be switched on	Computer is not receiving power. Check that the power cord is connected to the computer and to the wall power outlet.
SX-8G IP-Star shows no movement when a protocol is started	SX-8G IP-Star is not switched on. Check that the SX-8G IP-Star is switched on.
SX-8G IP-Star shows abnormal movement when a protocol is started	The pipettor head may have lost its home position. In the Software, select "Manual Operation/Home". After confirming that the pipettor head moves to the home position, run the protocol again.
Aspirated liquid drips from the disposable tips	Dripping is acceptable when ethanol is being handled. For other liquids: air is leaking from the syringe pumps. Grease or replace the O-rings. If the problem persists, contact DIAGENODE Technical Services.

Technical assistance

At DIAGENODE we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of DIAGENODE products. If you have any questions, or experience any difficulties regarding the SX-8G IP-Star or DIAGENODE products in general, do not hesitate to contact us.

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For technical assistance and more information call the DIAGENODE Technical Service Department or contact your local distributor.

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ASK THE EXPERTS

Please, do not hesitate to contact our customer support team if you have any questions about the design of your ChIP-seq experiment or the bioinformatics data analysis.

Contact for Europe, Asia, Oceania and Africa:
custsupport@diagenode.com
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Ordering information

Products	Cat. No. (new)	Cat. No. (old)	Format
IP-Star® Compact	B03000002	UH-002-0001	1 unit
Auto True MicroChIP kit x16	C01010140	/	16 rxns
Auto True MicroChIP & MicroPlex Library Preparation™ Package	C01010141	/	16 ChIP rxns & 12 library prep rxns
MicroPlex Library Preparation™ kit x12	C05010010	AB-004-0012	12 rxns
MicroPlex Library Preparation™ kit x48	C05010011	/	48 rxns
Auto Histone ChIP-seq kit protein A x16	C01010020	AB-Auto02-A016	16 rxns
Auto Histone ChIP-seq kit protein A x100	C01010022	AB-Auto02-A100	100 rxns
Auto Histone ChIP-seq kit protein G x16	C01010021	AB-Auto02-G016	16 rxns
Auto Histone ChIP-seq kit protein G x100	C01010023	AB-Auto02-G100	100 rxns
Auto iDeal ChIP-seq kit for Histones x 24	C01010057	/	24 rxns
Auto Transcription ChIP kit protein A x16	C01010030	AB-Auto03-A016	16 rxns
Auto Transcription ChIP kit protein A x100	C01010032	AB-Auto03-A100	100 rxns
Auto Transcription ChIP kit protein G x16	C01010031	AB-Auto03-G016	16 rxns
Auto Transcription ChIP kit protein G x100	C01010033	AB-Auto03-G100	100 rxns
Auto iDeal ChIP-seq kit for Transcription Factors x24	C01010058	/	24 rxns
Auto Plant ChIP-seq kit x24	C01010151	/	24 rxns
Auto MeDIP kit x16	C02010011	AF-Auto01-0016	16 rxns
Auto MeDIP kit x100	C02010012	AF-Auto01-0100	100 rxns
iDeal Library Preparation Kit x24 (incl. Index Primer Set 1)	C05010020	/	24 rxns
Auto hMeDIP kit x16	C02010033	AF-Auto02-0016	16 rxns
Auto MethylCap x48	C02020011	AF-Auto01-0048	48 rxns
Auto IPure kit v2 x100	C03010010	AL-Auto01-0100	100 rxns

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