diagenode

Innovating Epigenetic Solutions

AUTO IPure KIT v2 MANUAL

Magnetic DNA Purification kit for epigenetic applications

Auto IPure kit v2 x100

New Cat. No. C03010010, Old Cat. No. AL-Auto01-0100

Version 4 I 19_09_14

Technical Assistance & Ordering Information

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

Contents

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

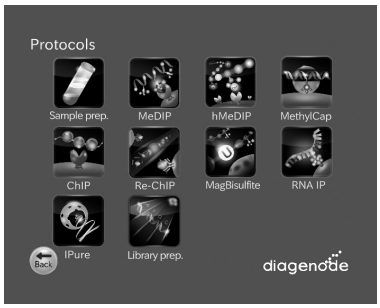
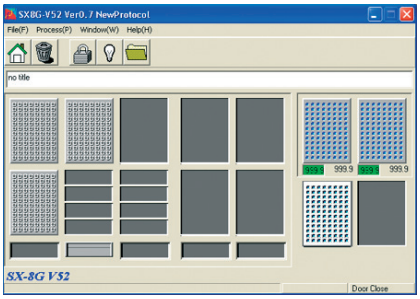
Diagenode has developed two automated platforms (IP-Star® and IP-Star® Compact) designed to increase your lab's productivity, efficiency and experimental reproducibility. The two automated platforms are capable of processing up to 16 samples per cycle. The automated systems processes sheared chromatin (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 (Auto ChIP kit, Auto Histone ChIP-seq kit, Auto Histone ChIP-seq kit, Auto MeDIP kit, Auto MethylCap kit, Auto hMeDIP, Auto IPure kit v2)
- 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 SX-8G 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 SX-8G 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 SX-8G IP-Star is designed to improve the accuracy and the reproducibility of any immunoprecipitiation experiment.

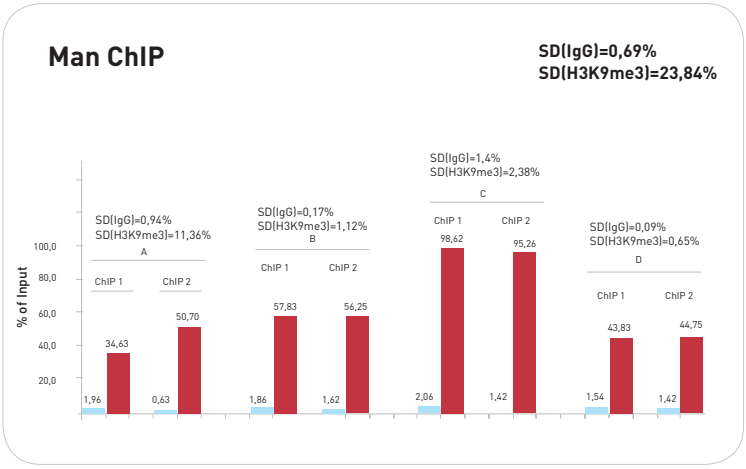


Figure 1: Manual ChIP. Four different operators have each performed two ChIP experiments using H3K9me3 antibody on the genomic region SAT2 (positive locus). 10,000 Hela cells have been used per IP. Reagents and sheared chromatin were identical per assay. The standard deviations between the ChIPs performed by the same operator and between the four different operators are displayed.

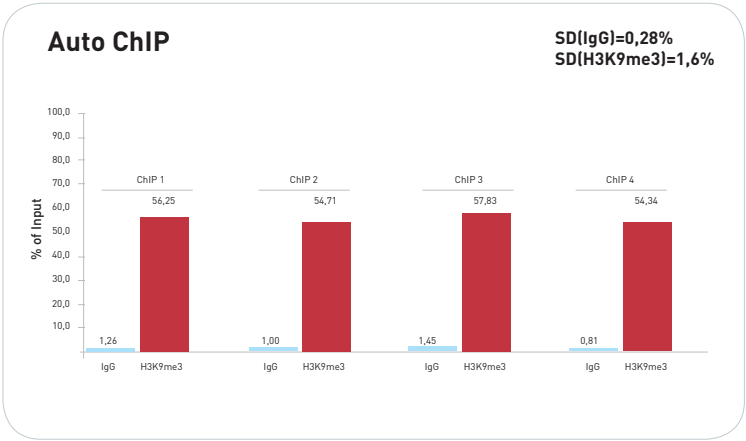
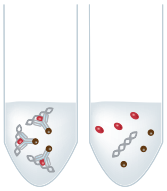


Figure 2: Automated ChIP. Four ChIP experiments using H3K9me3 antibody on the genomic region SAT2 (positive locus) have been performed by the SX-8G IP-Star. 10,000 Hela cells have been used per IP. Reagents and sheared chromatin were identical per assay. The standard deviations between the four ChIPs performed by the SX-8G IP-Star are displayed.

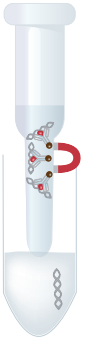
Kit Method Overview

IPure after ChIP



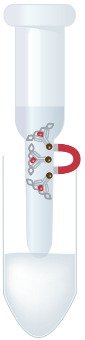
STEP 1.
Chromatin reverse cross-linking and elution

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.



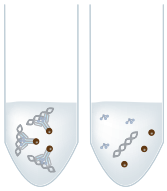
STEP 3. Washes

Proteins and antibody debris are washed away.

STEP 4. DNA Elution

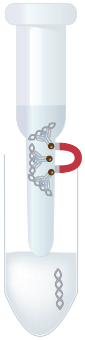
DNA is eluted from magnetic beads, which are discarded.
Purified DNA is ready for any downs-tream application (qPCR, next generation sequencing, amplification, microarray)

IPure after MeDIP



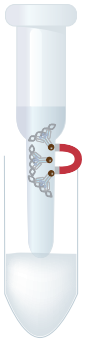
STEP 1.
DNA Elution

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 contents

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	15 ml	4°C
Buffer B	600 µl	4°C
Wash buffer 1 w/o iso-propanol	8 ml	4°C
Wash buffer 2 w/o iso-propanol	8 ml	4°C
Buffer C	8 ml	4°C
Magnetic beads	1.2 ml	4°C
Carrier*	300 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 Auto 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 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.

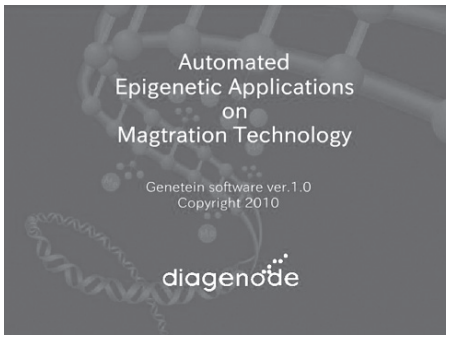
Wash Buffer 1

Wash buffer 1	100 rxns
Wash buffer 1 w/o iso-propanol	8 ml
Iso-propanol	8 ml
Total volume	**16 ml**

Wash Buffer 2

Wash buffer 2	100 rxns
Wash buffer 2 w/o iso-propanol	8 ml
Iso-propanol	8 ml
Total volume	**16 ml**

Running a protocol



Diagenode Splash Screen – A0

After the software start-up screen disappears, the Diagenode splash screen is displayed for several seconds, and then disappears.

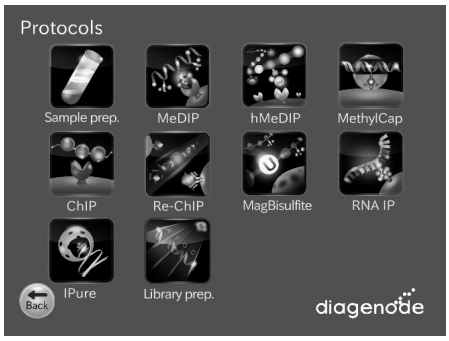
Start Screen – Top menu

After the Digenode splash screen disappears, the start screen is displayed. This is the first active window; it allows the user to enter into three different parts of the software.

USER ACTIONS:

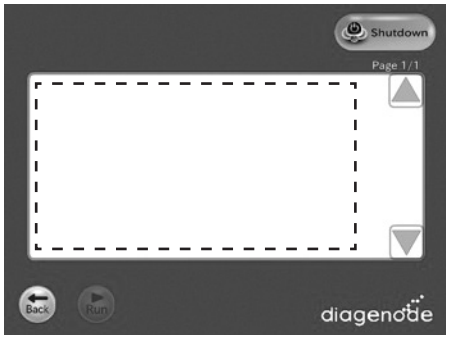
Buttons:

- Protocols
- Maintenance (for technical service)
- Information (Diagenode contact details)



Protocols screen

All available protocols are displayed on this screen.

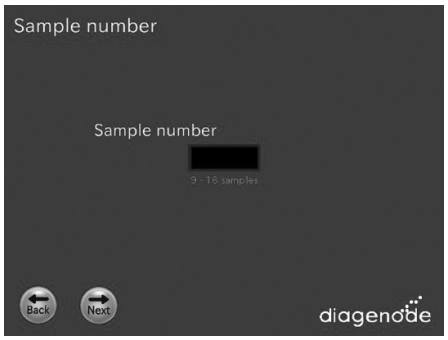


Screen – [Categories Name] Protocol List

After the user presses the "[Categories Name]" button, the "[Categories Name]" appears. When selected the protocol on the protocol list, the "Run" button shall turn executable.

Buttons:

- The user presses the "Back" button. The user returns to the "Protocols" screen.
- The user presses the "Shutdown" button. The screen shall be changed to "Power Off".
- The user presses the "Run" button. The screen shall be changed to "Sample number".
- ▲ Page up the list box.
- ▼ Page down the list box

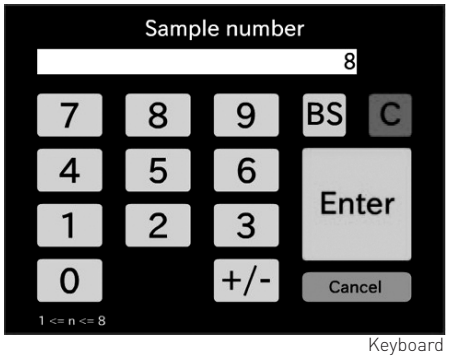


Screen – Sample number

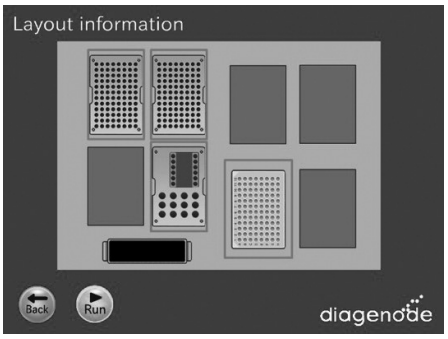
After the user presses the "Run" button, the "Sample number" appears.

Buttons:

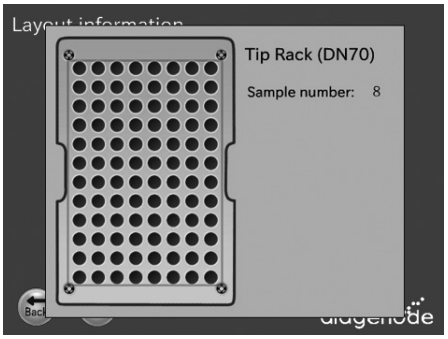
- The user presses the "Sample number" Text box. The screen will be changed to keyboard.
- The user presses the "Back" button. The user returns to the "Protocol List" screen.
- The user presses the "Next" button. The screen shall be changed to "Configuration" or "Layout information".



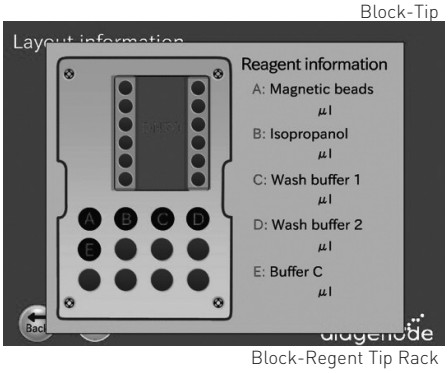
Keyboard



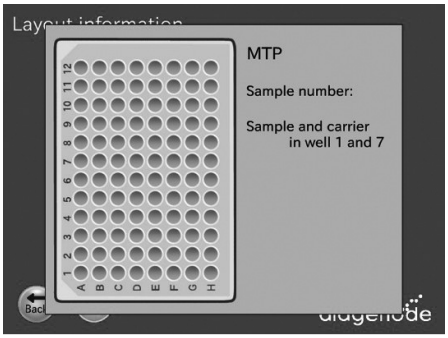
Layout information



Block-Tip



Block-Regent Tip Rack



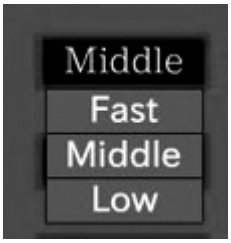
Block-PCR Tube

Screen – Layout Information

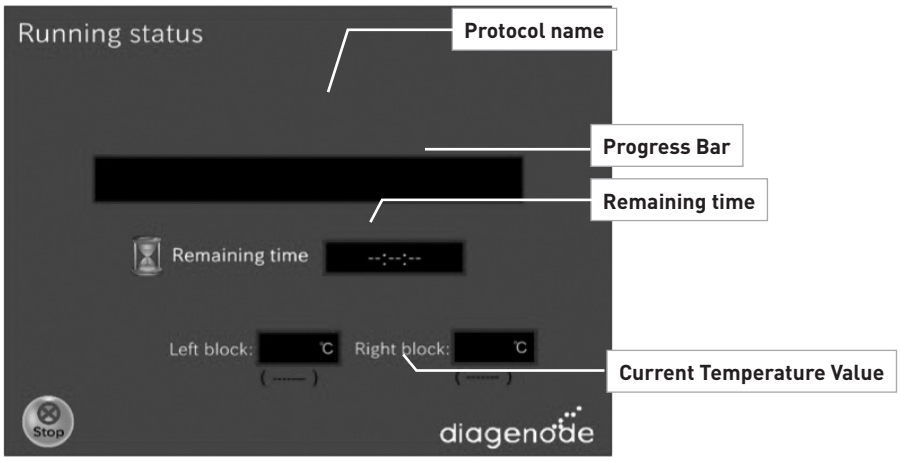
After the user presses the "next" button from "Sample number" screen or "Configuration" screen, the "Layout Information" screen appears.

Buttons:

- The user presses the "Back" button. The user returns to the previous screen.
- The user presses the "Next" button. The screen shall be changes to "Set confirmation".
- When the user presses a block, that block is magnified on the work surface layout background. The magnified view provides a better display of the correct method setup for that block on the work surface.
- Based on the selected protocol, the user follows the indications provided in the screens to set up correctly the different reagents and samples.



Speed list menu



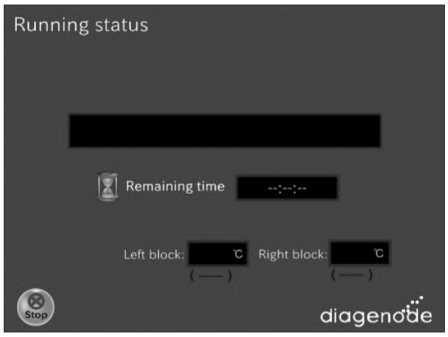
Screen – Running

After the user presses the "Run" button in the "Set confirmation" screen, the "Running" screen appears.

Buttons:

- The user presses the "Stop" button. Then screen shall be changed to "Stop Dialog".

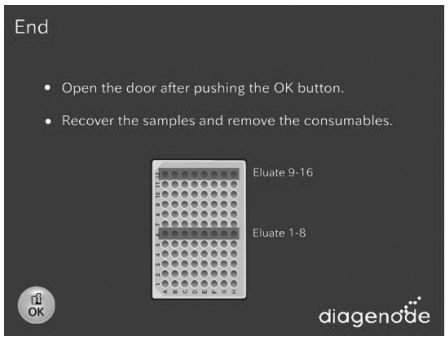
Status screen is preferred as a progress bar that moves across the screen as the step progresses



Screen – Running status

This screen gives informations about the current running step of the protocol.

The user can check trough this screen the passed and remaining time of the experiment.



Screen – Finish/End

When the protocol is complete, a window appears telling user the run is over. The screen behind this window should be the Startup screen. When OK is pressed, then the Startup screen appears and the user can immediately begin to remove their sample and prepare for the next run.

At this point, user is expected to be ready to press RUN.

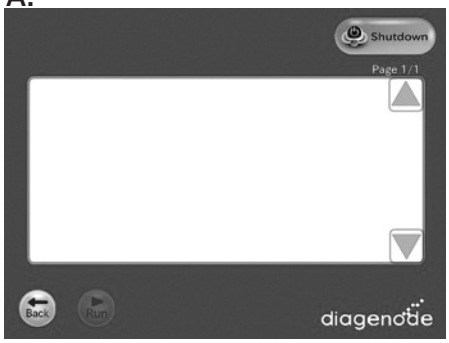
Buttons:

- The user presses the "OK" button. Then screen shall be changed to "[Categories Name] Protocol List".

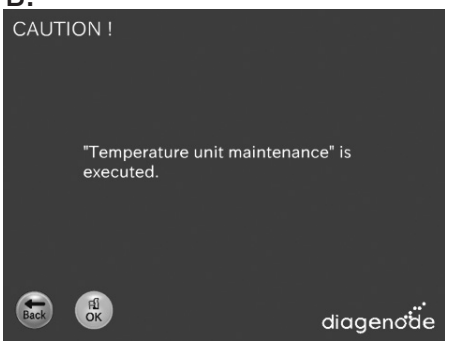
Screen – Caution !

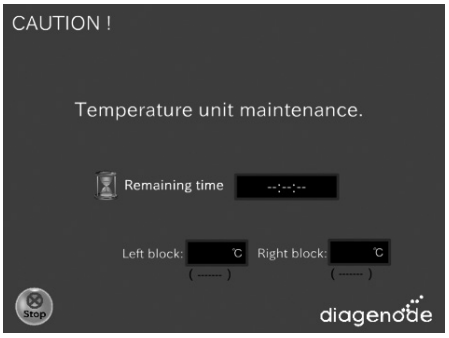
When the protocol finishes the user can return to the protocol list (screen **A.**) or warm the peltier block (screen **B.**) to eliminate possible condensation in the block.

A.



B.





IP-STAR®

How to perform Auto IPure on the IP-Star®

How to perform Automated IPure on the IP-Star®

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

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 v2 buffers

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

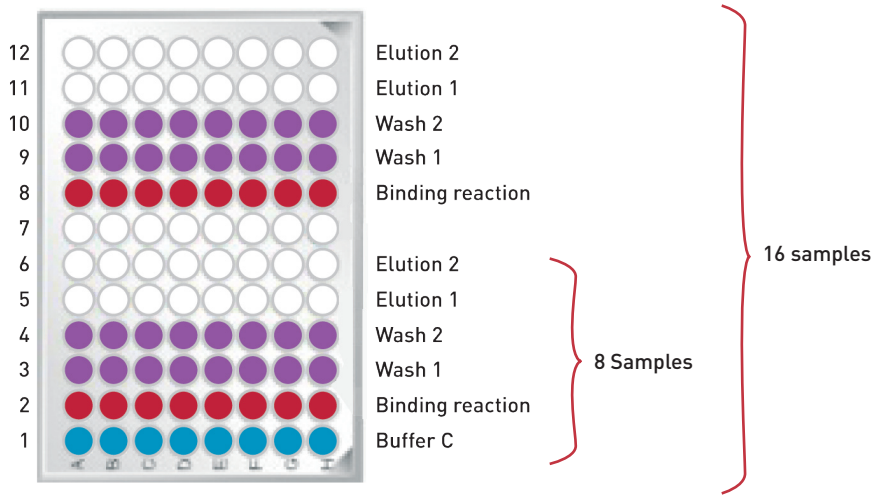
Wash Buffer 1

Wash buffer 1	100 rxns
Wash buffer 1 w/o iso-propanol	8 ml
Iso-propanol	8 ml
Total volume	**16 ml**

Wash Buffer 2

Wash buffer 2	100 rxns
Wash buffer 2 w/o iso-propanol	8 ml
Iso-propanol	8 ml
Total volume	**16 ml**

C) Dispense prepared reagents in 96 well plate



Well	Description	Volumes
12	Buffer C to be dispensed by the IP-Star	25 µl
11	Buffer C to be dispensed by the IP-Star	25 µl
10	Wash Buffer 2	100 µl
9	Wash Buffer 1	100 µl
8	Isopropanol + beads + carrier	100 µl + 10 µl + 2 µl + 100 µl
7	empty	
6	Buffer C to be dispensed by the IP-Star	25 µl
5	Buffer C to be dispensed by the IP-Star	25 µl
4	Wash Buffer 2	100 µl
3	Wash Buffer 1	100 µl
2	Isopropanol + beads + carrier + sample	100 µl + 10 µl + 2 µl+100 µl
1	Buffer C	150 µl

Alternatively, elution can be done in 150 µl followed by ethanol precipitation if final volume must be reduced.

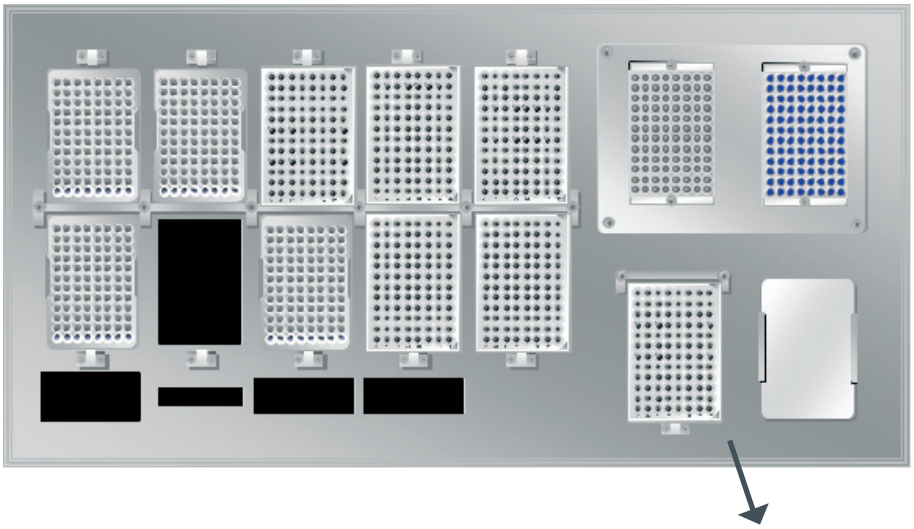
Sample can be IP sample or input

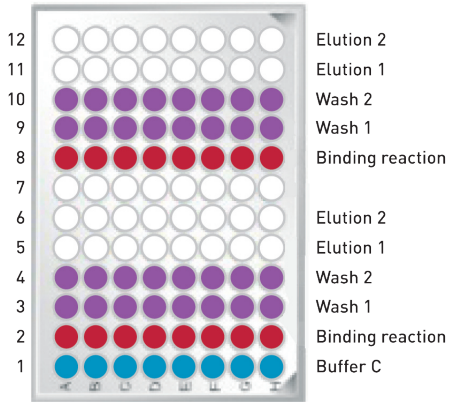
D) Loading and running protocol

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**

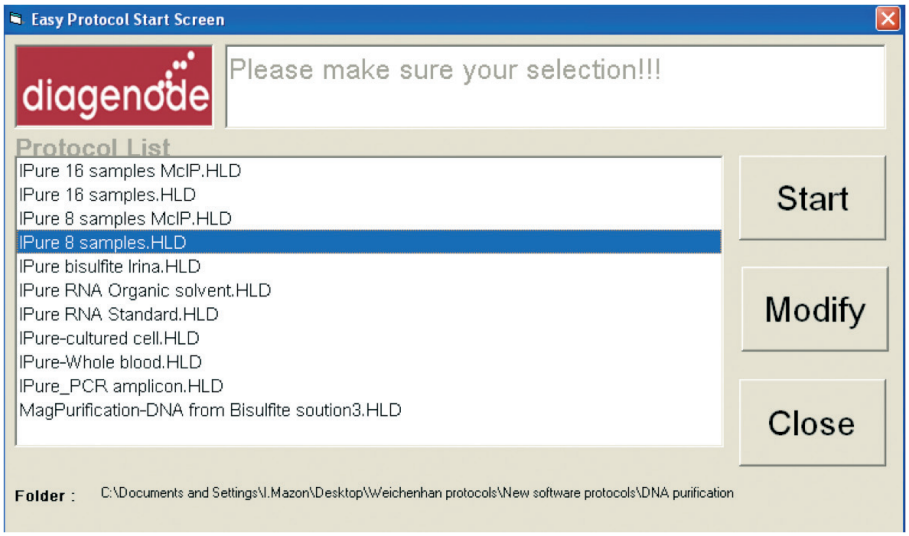
1. Switch on the SX-8G IP Star. The power switch is on the right side of the instrument.
2. Switch on the computer. 
3. Start SX-8G V52 software through SX-8G V52 the following icon
4. Place the prepared 96 well plate in the indicated room temperature module in the workstation



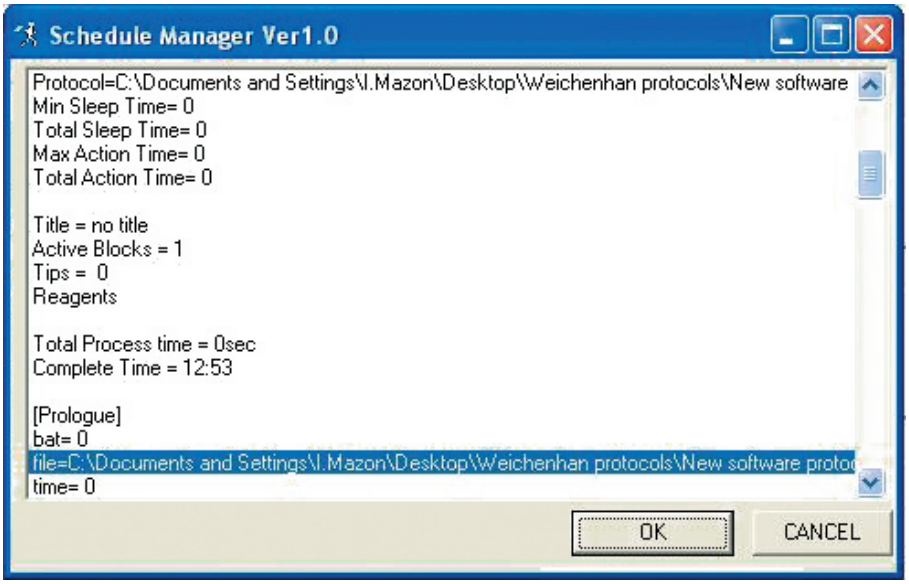


5. Press the following icon 

   Select the protocol of interest. Press start.

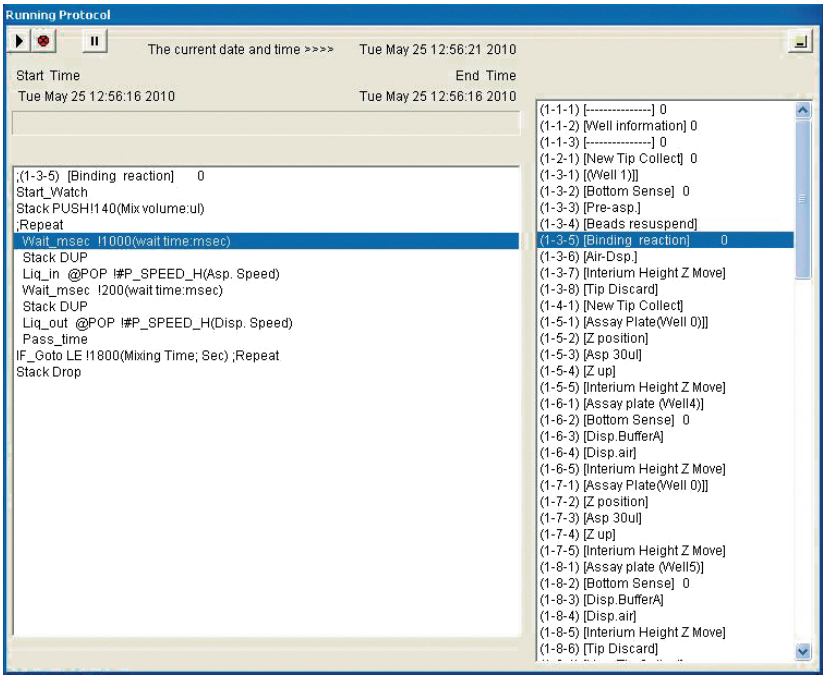


6. Before starting the protocol a start confirmation window will appear. Press OK and the protocol will run.

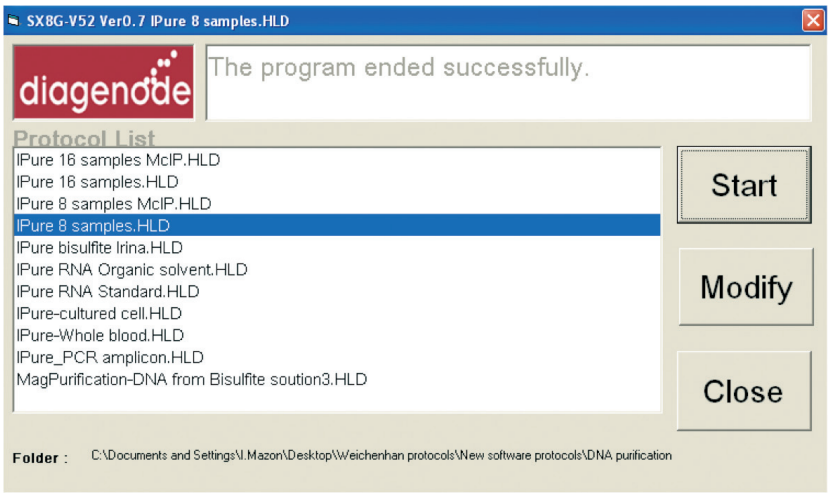


7. The program will run through the following steps: magnetic bead washes, IP and IP washes.

   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

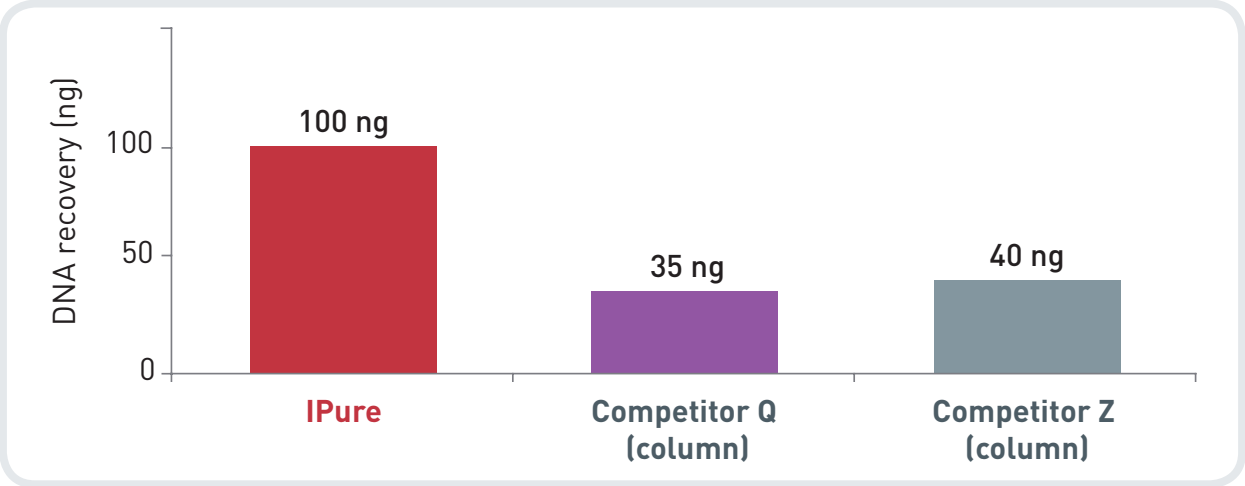
Shutting down the IP-Star

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!

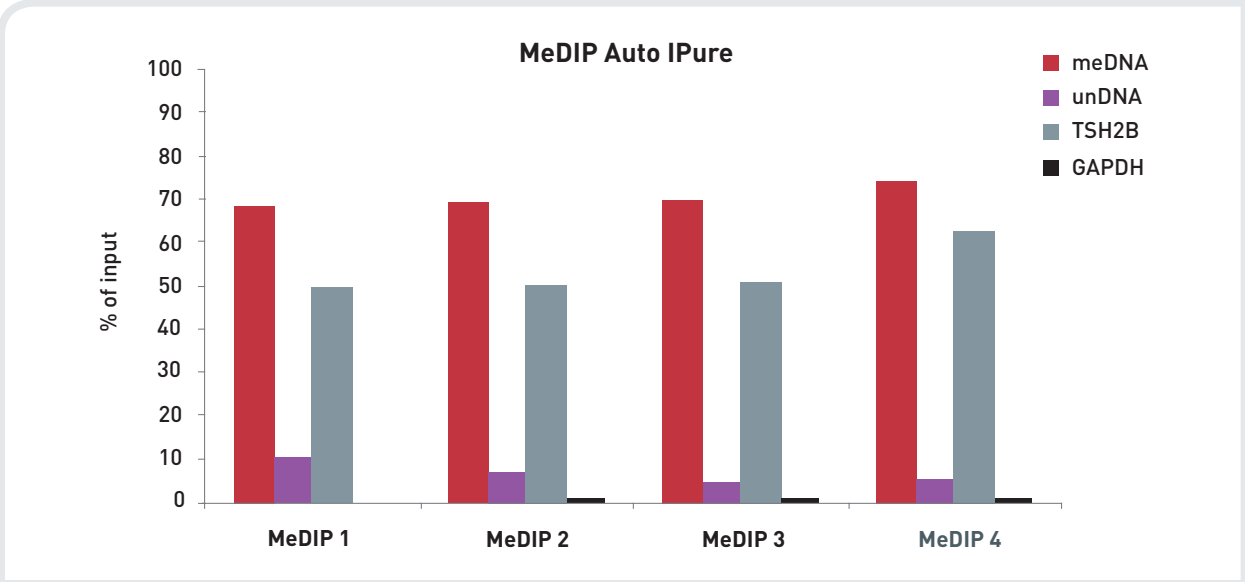
Results

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



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 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.

DIAGENODE customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at DIAGENODE. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

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

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Ordering information

Description	Cat. No. (NEW)	Cat. No. (OLD)	Format
IP-Star® Compact	B03000002	UH-002-0001	1 unit
Auto True MicroChIP kit	C01010140	/	16 rxns
Auto True MicroChIP & MicroPlex Library Prep Package	C01010141	/	16 ChIP rxns & 12 library prep rxns
MicroPlex Library Preparation kit x12	C05010010	AB-004-0012	12 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 prowwtein G x16	C01010021	AB-Auto02-G016	16 rxns
Auto Histone ChIP-seq kit protein G x100	C01010023	AB-Auto02-G100	100 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 ChIP kit protein A x100	C01010011	AB-Auto01-A100	100 rxns
Auto ChIP kit protein G x100	C01010013	AB-Auto01-G100	100 rxns
Auto MeDIP kit x16	C02010011	AF-Auto01-0016	16 rxns
Auto MeDIP kit x100	C02010012	AF-Auto01-0100	100 rxns
Auto hMeDIP kit x16	C02010033	AF-Auto02-0016	16 rxns
Auto MethylCap x48	C02020011	AF-Auto01-0048	48 rxns
Auto IPure kit v2	C03010010	AL-Auto01-0100	100 rxns

Visit us at one of Diagenode's demo sites or discover our Automated Systems by performing some assays with the help of our R&D and Technical Department.

www.diagenode.com

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