diagenode

A Hologic Company

AUTO MagMeDIP KIT MANUAL

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Technical Assistance & Ordering Information

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http://www.diagenode.com/en/company/distributors.php
For the rest of the world, please contact Diagenode s.a.

Contents

Introduction

The Diagenode SX-8G 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 MagMeDIP kit

The Diagenode Auto MagMeDIP kit is designed to perform automated Immunoprecipitate of methylated DNA using the SX-8G IP-Star.

The Auto MagMeDIP kit contains the antibody directed against 5-methyl Cytidine as well as meDNA and unDNA internal IP controls. The IP has been optimized to specifically select and precipitate the methylated DNA: by the use of our antibody, buffers and protocol. The IP efficiency can indeed be double-checked with the use of our internal controls. Furthermore, the use of the Automated System will drastically increase the consistency of your MeDIP Assay.

The Auto MagMeDIP kit allows you to perform DNA methylation analysis of your sample together with optimized internal IP controls: ALL IN ONE TUBE. The methylated DNA (meDNA) and unmethylated DNA (unDNA) controls allow for direct CORRELATION between IP'd MATERIAL and METHYLATION STATUS. This methylation analysis is FAST, HIGHLY SPECIFIC and each IP is QUALITY controlled: essential keys for RELIABLE results.

In the Auto MagMeDIP kit, the protocol has been improved to allow researchers to work in smaller tubes than traditionally did so far. The kit ensures the use of low amount of reagents per reaction (not only antibodies, but also buffers) and it includes fewer buffers in comparison with other kits.

The Kit provides you with a DNA isolation buffer for an extra-fast method to purify your IP'd material (for qPCR analysis). Alternatively, for other applications, e.g. sequencing, linear amplification or microarray. Magnetic DNA purification (IPure) protocols can be used as alternative.

Combination of this High Quality Kit and the SX-8G IP-Star Robot will allow you to perform DNA Methyaltion Profiling in less than 9 hours! Starting with sheared DNA the Automated System will provide you with the purified methylated DNA of your sample.

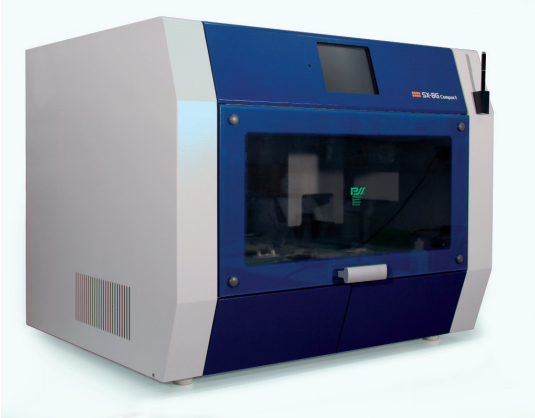
The Auto MagMeDIP kit protocol has been validated using genomic DNA sheared by sonication using the Bioruptor®.

SX-8G IP-Star® and SX-8G IP-Star® Compact Systems for automation of epigenetic applications

Diagenode has developed two automated platforms (SX-8G IP-Star® and SX-8G 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 SX-8G IP-Star® and SX-8G 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.

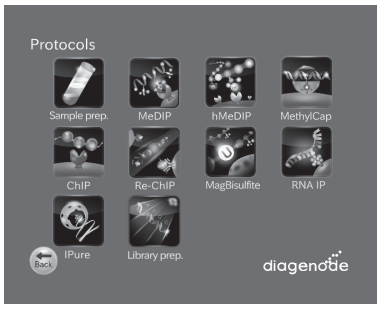
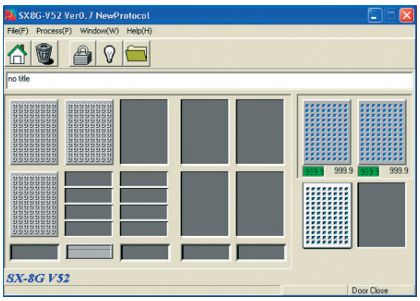
Major benefits of Diagenode Automated Platforms

SX-8G IP-Star® Compact



SX-8G IP-Star®

- → Increased reproducibility
- → A consistency "Walk-away research" (less "hands-on-work")
- → Huge time savings
- → Easy and flexible programming
- → Validated for ChIP, MeDIP & MBD
- → Compatible with Diagenode Kits (Auto ChIP kit, Auto Histone ChIP-seq kit, Auto Transcription ChIP kit, Auto MagMeDIP kit, Auto MethylCap kit, Auto hMeDIP kit, Auto IPure kit)
- → Reduces cross-contamination

	SX-8G IP-Star® Compact	SX-8G 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.

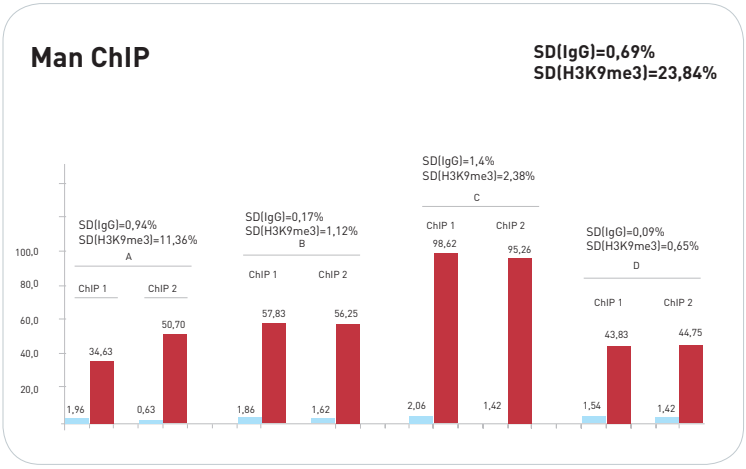


Figure 1: Manual ChIP. Four different operators have each performed two ChIP experiments using H3K9me3 antibody on the genomic region SAT2 (positive locus). 10,000 Hela cells have been used per IP. Reagents and sheared chromatin were identical per assay. The standard deviations between the ChIPs performed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

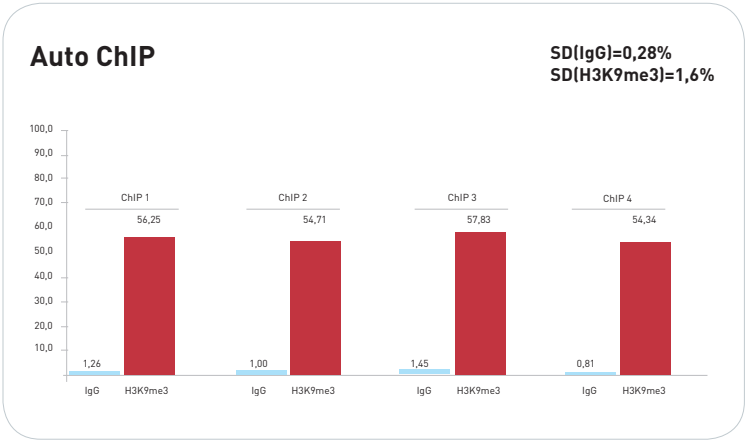


Figure 2: Automated ChIP. Four ChIP experiments using H3K9me3 antibody on the genomic region SAT2 (positive locus) have been performed by the SX-8G IP-Star. 10,000 Hela cells have been used per IP. Reagents and sheared chromatin were identical per assay. The standard deviations between the four ChIPs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

Kit Method Overview

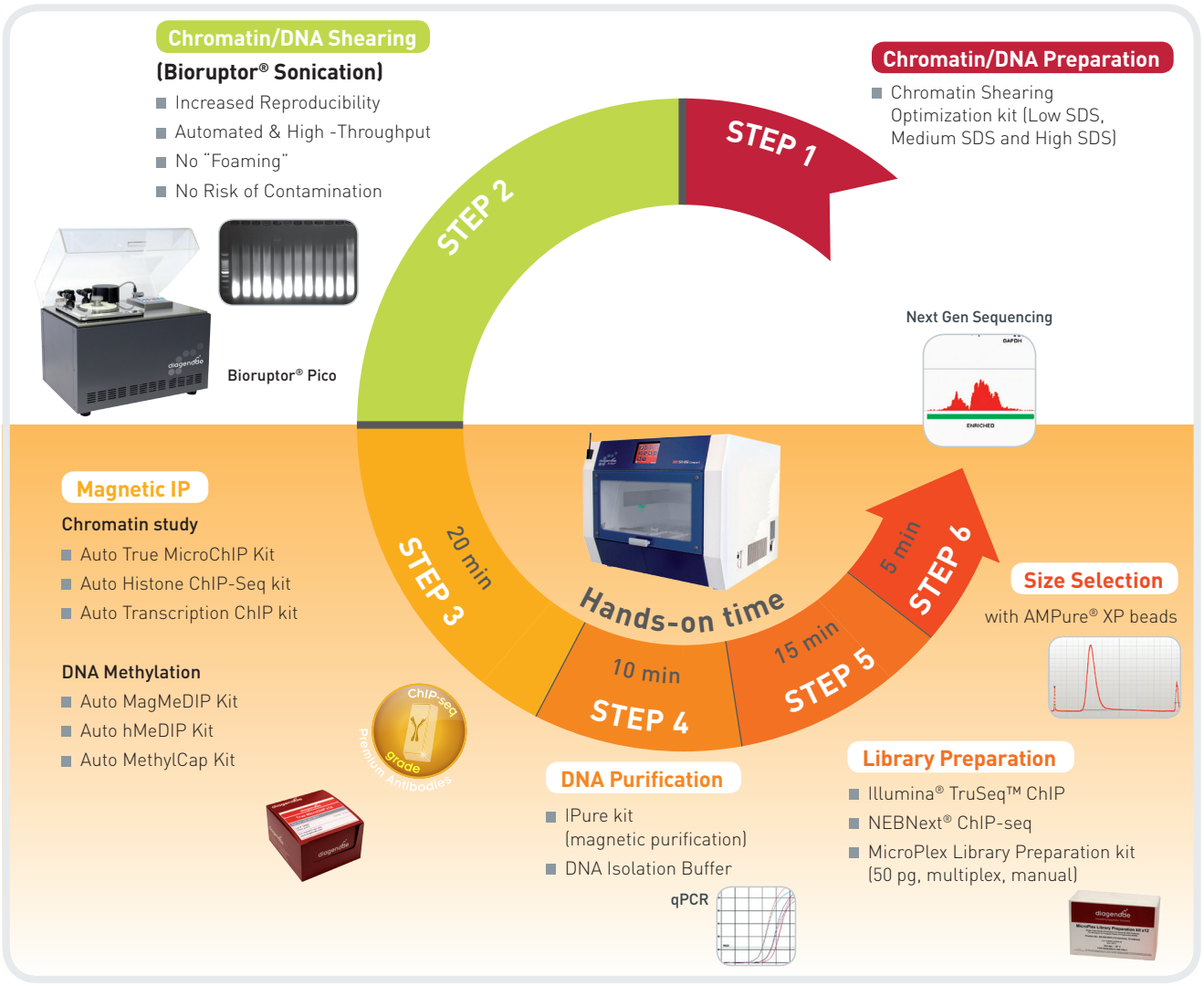


Figure 3. Diagenode provides a full suite of automated solutions for ChIP experiments.

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.

Kit Materials

Kit Content

Two Auto MagMeDIP Kit formats are available and the kit content is sufficient to perform either 10 or 48 MagMeDIP assays by using the SX-8G IP-Star Automated System. The kit content is described in Table 1. Upon receipt, store the components at the temperatures indicated in Table.

Table 1 (Note: Upon receipt, store the components at the right temperature)

GenDNA Module			
Component	Description	Quantity (x10 / x48)	Storage
GenDNA Digestion buffer	Detergent, salt and ion chelator included	3 ml	4°C
GenDNA Proteinase K (200x)	200x stock solution	300 µg / 15 µl	-20°C
GenDNA precipitant	Salt included	3 ml	4°C
GenDNA TE	Ion chelator included	3 ml	4°C
GenDNA RNAse (DNAse free)	-	5 µg / 10 µl	-20°C

Table 2

Methylated DNA IP Module				
Component	Description	Quantity (x10)	Quantity (x48)	Storage
Magbeads	Blocker and 0.02% sodium azide included.	150 µl	750 µl	4°C, Do not freeze
Water	-	2x 2 ml	10 ml	4°C
MagBuffer A	Detergent mix, salt and **ion chelator mix included**	2 ml	10 ml	4°C
MagBuffer B	-	100 µl	500 µl	4°C
MagBuffer C	-	40 µl	200 µl	-20°C
Antibody anti-5mC*	5-methylcytosine antibody	5 µl	25 µl	-80°C
meDNA Positive control	Methylated DNA control	20 µl	20 µl	-20°C
unDNA Negative control	Unmethylated DNA control	20 µl	20 µl	-20°C
MagWash buffer-1	Detergent mix, salt and ion chelator mix included.	6 ml	30 ml	4°C
MagWash buffer-2	Ion chelator mix included.	4 ml	20 ml	4°C
DNA isolation Buffer (DIB)	-	4 ml	20 ml	4°C
Proteinase K	100x stock solution	20 µl	200 µl	-20°C
Primer pair #1 (meDNA control)	5 µM each (Rv & Fw)	50 µl	50 µl	-20°C
Primer pair #2 (unDNA control)	5 µM each (Rv & Fw)	50 µl	50 µl	-20°C
hum meDNA primer pair (TSH2B)	5 µM each (Rv & Fw)	50 µl	50 µl	-20°C
hum unDNA primer pair (GAPDH)	5 µM each (Rv & Fw)	50 µl	50 µl	-20°C
200-µl tube strips	-	2	8	RT
Cap strips	-	2	8	RT

Table 3. Components available separately

Description	Reference	Quantity	Storage
200 µl tube strips (12 tubes/strip) + cap strips	WA-001-0080	80	RT
200 µl tube strips (8 tubes/strip) + cap strips for SX-8G IP-Star® Compact	WA-002-0120	120	RT
Tips (bulk)	WC-001-1000	1000	RT
Tips (box)	WC-002-0960	10x96	RT

Table 4. Kits and Modules available separately

Description	Reference	Quantity
Chromatin shearing optimization kit - Low SDS	C01020010 (AA-001-0100)	1 kit
Chromatin shearing optimization kit - Medium SDS	C01020011 (AA-002-0100)	1 kit
Chromatin shearing optimization kit - High SDS	C01020012 (AA-003-0100)	1 kit
IPure	AL-100-0100	100 rxns
Auto IPure	AL-Auto01-0100	100 rxns

Table 5. Plastics and consumables available separately

Description	Reference	Quantity
200 µl tube strips (12 tubes/strip) + cap strips	WA-001-0080	80 pc
200 µl tube strips (8 tubes/strip) + cap strips for SX-8G IP-Star® Compact	WA-002-0120	120 pc
96 well microplates	WA-003-0010	10 pc
Tips (box)	WC-002-0960	960 pc
Tips (bulk)	WC-001-1000	1000 pc
2 ml microtube for SX-8G IP-Star® Compact	WA-008-0100	100 pc
Large reagent container for SX-8G IP-Star® Compact	WA-007-0020	20 pc
Medium reagent container for SX-8G IP-Star® Compact	WA-006-0010	10 pc

How to perform Automated MagMeDIP in the SX-8G IP-Star® Compact

A. Prepare Reagents

1. Preparation of MagBuffer A (1x) (for 1 IP) in 1.5 ml tube.

MagBuffer (1x)	200 µl
MagBuffer A (5x)	40 µl
Water	160 µl

B. Prepare Antibody and IP Mixes

2. Preparation of Antibody Dilution (1:2) (max. 6 IP) in 1.5 ml tube.

Antibody (1:2)	2 µl
Antibody	1 µl
Water	1 µl

3. Preparation of Antibody Mix in 1.5 ml tube.

Antibody Mix	One IP	For 2 IPs	For 4 IPs	For 6 IPs	For 8 IPs
Antibody 1:2	0.30 µl	0.75 µl	1.50 µl	2.00	3.00 µl
MagBuffer A (5x)	0.60 µl	1.50 µl	3.00 µl	4.00	6.00 µl
Water	2.10 µl	5.25 µl	10.50 µl	14.00	21.00 µl
MagBuffer C	2.00 µl	5.00 µl	10.00 µl	13.00	20.00 µl
FINAL VOLUME	5.00 µl	12.50 µl	25.00 µl	33.00	50.00 µl

4. Preparation of Incubation Mix (1 IP + 1 Input) in 1.5 ml tube.

Incubation Mix	90 µl
H_2O	45 µl
MagBuffer A (5x)	24 µl
MagBuffer B	6 µl
meDNA positive control	1.5 µl
unDNA negative control	1.5 µl
Sheared DNA (0.1 µg/µl)	12 µl

a. Incubate at 95°C for 3 minutes

b. Quickly chill on ice (it is best to use ice-water)

c. Perform a short spin at 4°C.

Note: The incubation mix is prepared in excess. 75 µl will be needed for the IP.

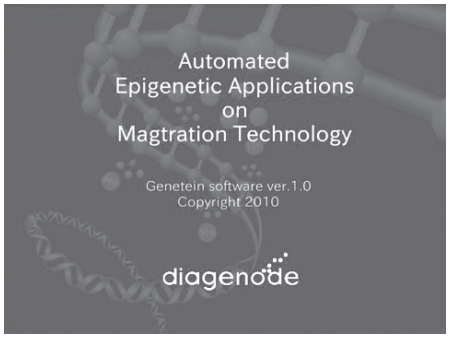
d. Take 7.5 µl incubation mix and store it in a new tube. This is the input.

Before dispensing, mix the content in the tube by pippeting up and down.

5. Preparation of Immunoprecipitation Mix (for 1 IP) in 1.5 ml tube.

Immunoprecipitation Mix	100 µl
MagBuffer A (1x)	20 µl
Incubation Mix	75 µl
Antibody Mix	5 µl

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.

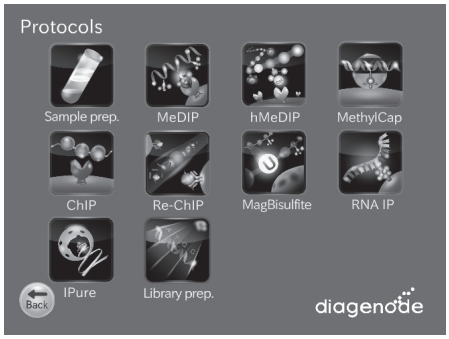
Start Screen – Top menu

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:

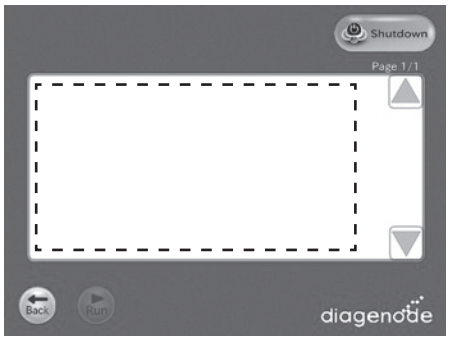
Buttons:

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



Protocols screen

All available protocols are displayed on this screen.

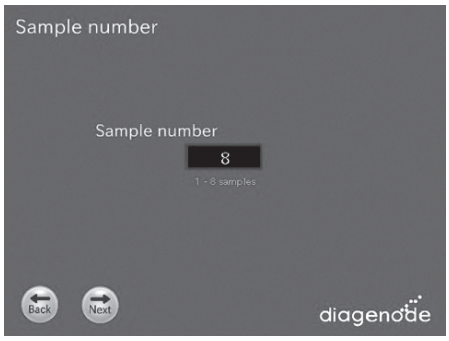


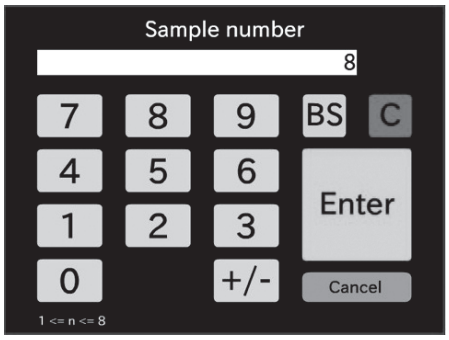
Screen – [Categories Name] Protocol List

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





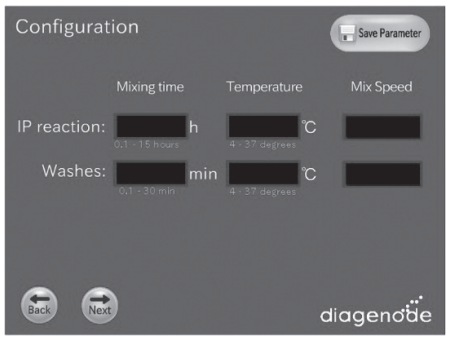
Keyboard

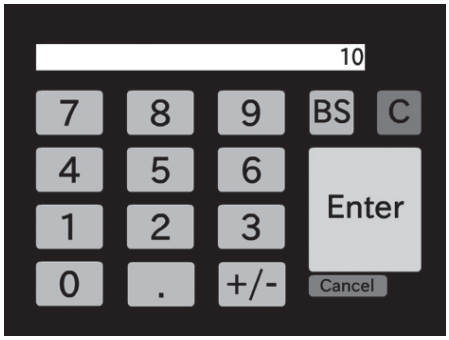
Screen – Sample number

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

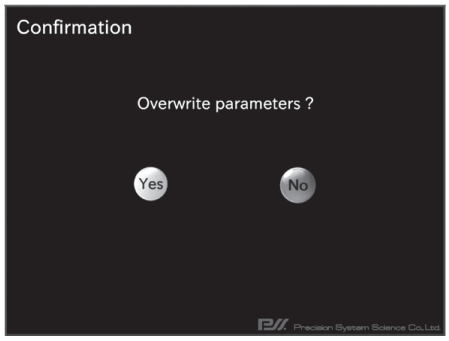
Buttons:

- 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



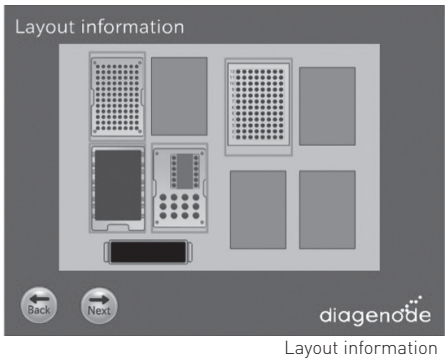
Screen – Configuration

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

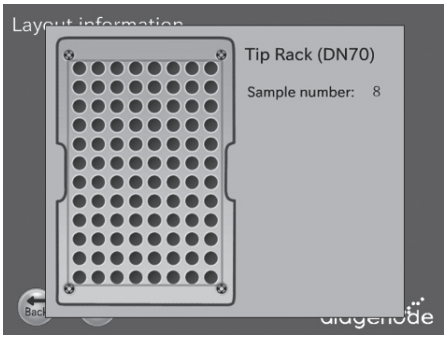
Buttons:

- 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 "Layout information".
- The user presses the "Save Parameter" button. The screen will be changed to "Save Parameter - Confirmation".
  - OK – Current parameters shown in the Display View will be stored to the [Protocol].ptd. And, returns the user to the display of the "Configuration" screen.
  - No – Returns the user to the display of the "Configuration" screen.
- The user presses the Text box. The screen will be changed to Keyboard or Speed list menu.

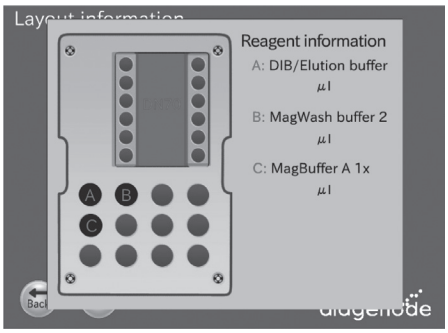
Speed list menu



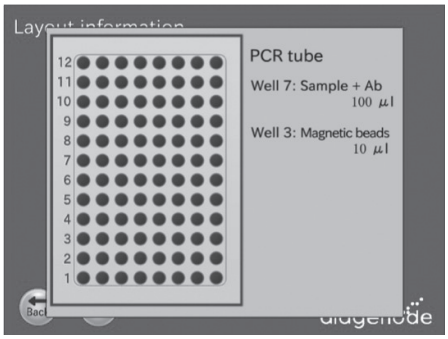
Layout information



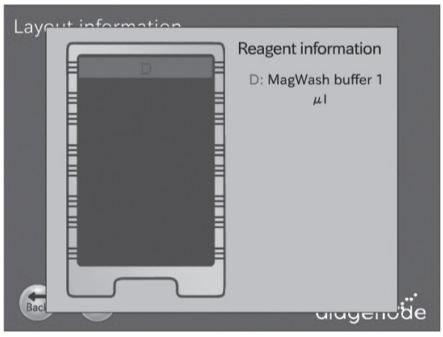
Block-Tip



Block-Regent Tip Rack



Block-PCR Tube

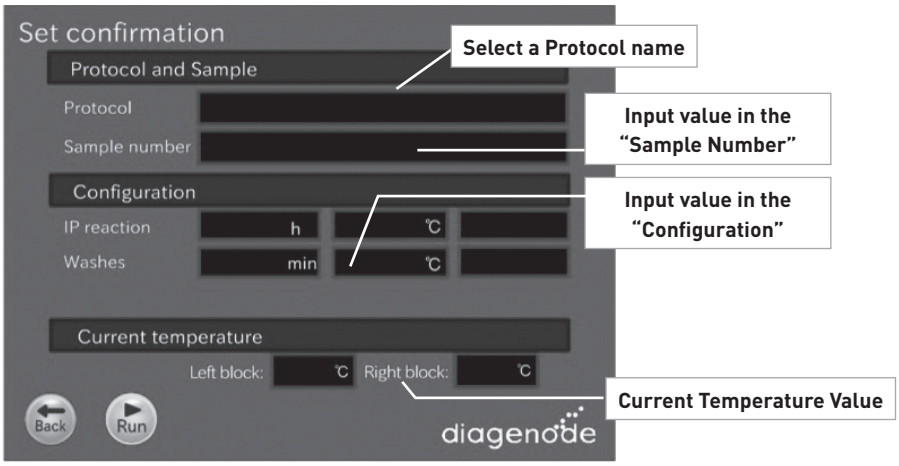


Screen – Layout Information

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 changes to "Set confirmation".
- When the user presses a block, that block is magnifies 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 based on the selected ChIP protocol.



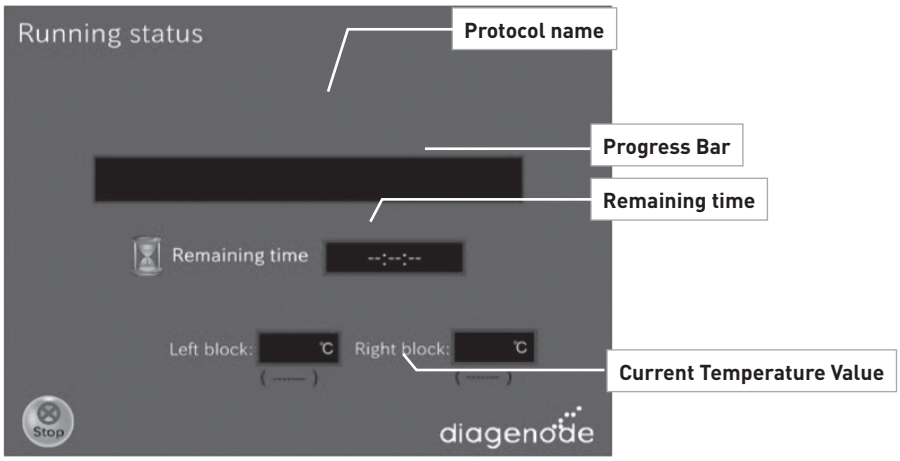
Screen – Set confirmation

After the user presses the "next" button in the "Layout information" screen, the "Set confirmation" screen appears.

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

Buttons:

- The user presses the "Back" button. The user returns to the layout information screen.
- The user presses the "Run" button. This is the expected action when user gets to this display after reviewing blocks. Runs the protocol.



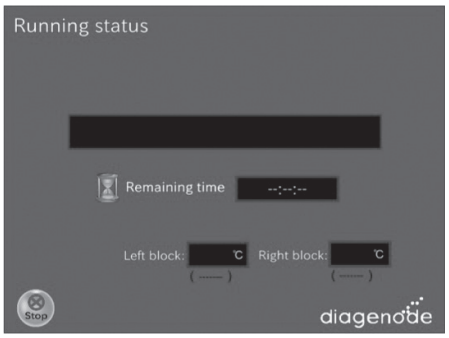
Screen – Running

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

Buttons:

- The user presses the "Stop" button. The screen changes to "Stop Dialog".

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

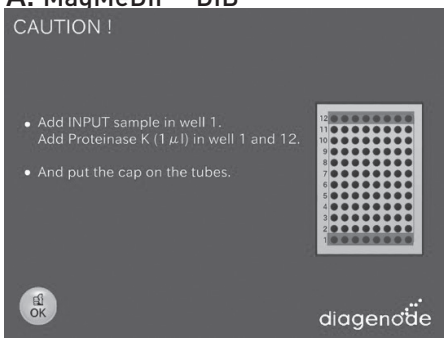


Screen – Running status

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

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

A. MagMeDIP - DIB



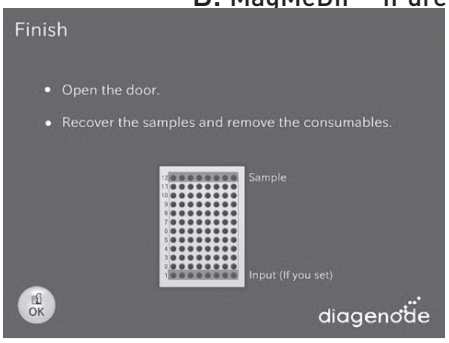
Screen – Elution

INPUT is defined as

INPUT= 7.5µl Incubation Mix + 92.5 µl DIB buffer

***IMPORTANT**: Please note that the enriched methylated DNA in DIB buffer is single strand DNA that can be directly analyzed by qPCR. For downstream applications such as sequencing or arrays, the enriched methyated DNA needs to be purified by phenol/chroloform extraction and converted to double stranded DNA.*

B. MagMeDIP - IPure

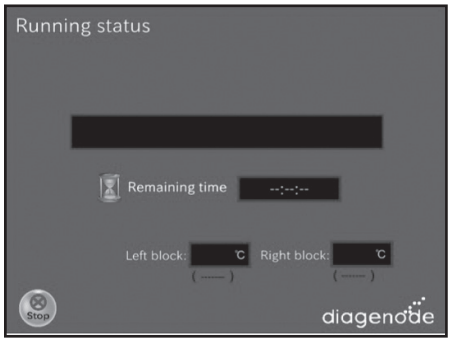


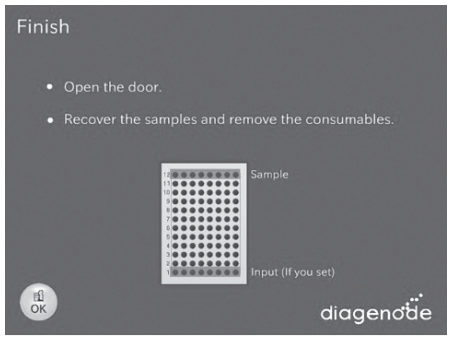
Screen – Elution

INPUT is defined as

INPUT= 7.5 µl Incubation Mix + 92.5 µl Elution Buffer (IPure kit)

***IMPORTANT**: Please note that the enriched methylated DNA is single stranded DNA that needs to be purified before analysis. DNA can be purified following IPure automated protocols and Diagenode's Auto IPure kit (see Auto IPure kit user manual) Alternatevely, the enriched methylated DNA could be purified by phenol/chroloform extraction or using spin columns. For downstream applications such as sequencing or arrays, the enriched methyated DNA needs to be converted to double stranded DNA.*





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 the next run.

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

Buttons:

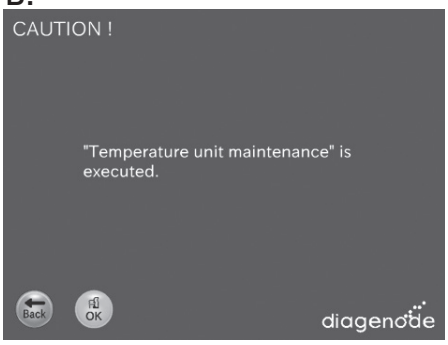
- The user presses the "OK" button. Then screen shall be changed to "[Categories Name] Protocol List".

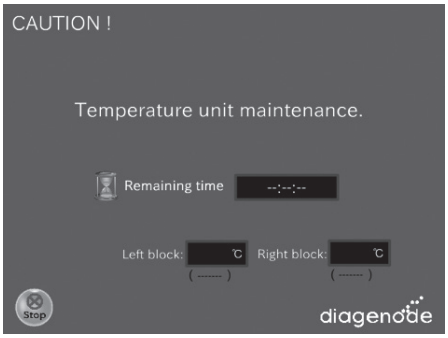
Screen – Caution !

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.





How to perform Automated MagMeDIP in the SX-8G IP-Star®

A) Prepare Reagents

1. Preparation of MagBuffer A (1x) (for 1 IP) in 1.5 ml tube.

MagBuffer (1x)	200 µl
MagBuffer A (5x)	40 µl
Water	160 µl

B) Prepare Antibody and IP Mixes

2. Preparation of Antibody Dilution (1:2) (max. 6 IP) in 1.5 ml tube.

Antibody (1:2)	2 µl
Antibody	1 µl
Water	1 µl

3. Preparation of Antibody Mix in 1.5 ml tube.

Antibody Mix	One IP	For 2 IPs	For 4 IPs	For 6 IPs	For 8 IPs
Antibody 1:2	0.30 µl	0.75 µl	1.50 µl	2.00	3.00 µl
MagBuffer A (5x)	0.60 µl	1.50 µl	3.00 µl	4.00	6.00 µl
Water	2.10 µl	5.25 µl	10.50 µl	14.00	21.00 µl
MagBuffer C	2.00 µl	5.00 µl	10.00 µl	13.00	20.00 µl
FINAL VOLUME	5.00 µl	12.50 µl	25.00 µl	33.00	50.00 µl

4. Preparation of Incubation Mix (1 IP + 1 Input) in 1.5 ml tube.

Incubation Mix	90 µl
H_2O	45 µl
MagBuffer A (5x)	24 µl
MagBuffer B	6 µl
meDNA positive control	1.5 µl
unDNA negative control	1.5 µl
Sheared DNA (0.1 µg/µl)	12 µl

a. Incubate at 95°C for 3 minutes

b. Quickly chill on ice (it is best to use ice-water)

c. Perform a short spin at 4°C.

Note: The incubation mix is prepared in excess. 75 µl will be needed for the IP.

d. Take 7.5 µl incubation mix and store it in a new tube. This is the input.

Before dispensing, mix the content in the tube by pippeting up and down.

5. Preparation of Immunoprecipitation Mix (for 1 IP) in 1.5 ml tube.

Immunoprecipitation Mix	100 µl
MagBuffer A (1x)	20 µl
Incubation Mix	75 µl
Antibody Mix	5 µl

C) Dispense prepared reagents into the corresponding tubes (see picture below)

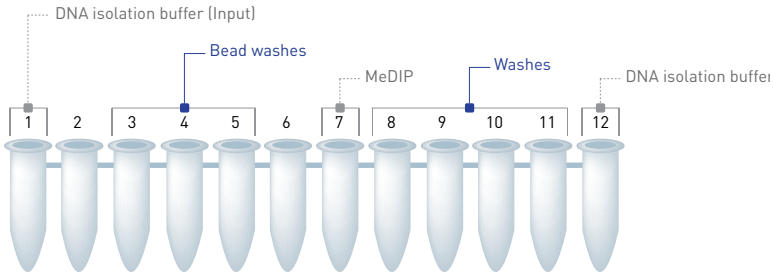
Note: Reagents dispension is different depending on the DNA purification method selected.

Loading reagents: make sure that all reagents are in the bottom of the tubes (especially magnetic beads) before starting the protocol.

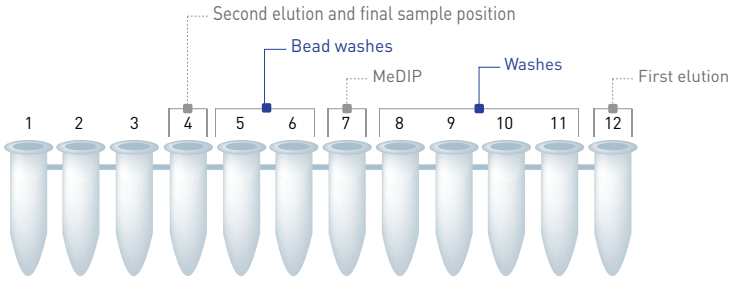
		DIB		IPURE
Tube #	Description	Volume	Description	Volume
1	DNA isolation buffer	92.5 µl	-	-
2	Empty		-	-
3	Magnetic beads	10 µl	-	-
4	MagBuffer A (1x)	50 µl	Elution buffer (IPure kit)	50 µl
5	MagBuffer A (1x)	50 µl	MagBuffer A (1x) + beads	50 µl + 10 µl
6	-		MagBuffer A (1x)	50 µl
7	Immunoprecitation Mix	100 µl	Immunoprecitation Mix	100 µl
8	MagWash buffer-1	100 µl	MagWash buffer-1	100 µl
9	MagWash buffer-1	100 µl	MagWash buffer-1	100 µl
10	MagWash buffer-1	100 µl	MagWash buffer-1	100 µl
11	MagWash buffer-2	100 µl	MagWash buffer-2	100 µl
12	DNA isolation buffer	100 µl	Elution buffer (IPure kit)	50 µl

1. For IPure method, Input sample will be purified following the IPure kit instructions.
2. For MeDIP-IPure experiments See intructions in the Auto IPure manual to prepare the Elution Buffer (Buffer A+ B)

DIB



IPure



IMPORTANT: At the end of the MagMeDIP-IPure reaction, 100 µl of IP sample are collected from well 4

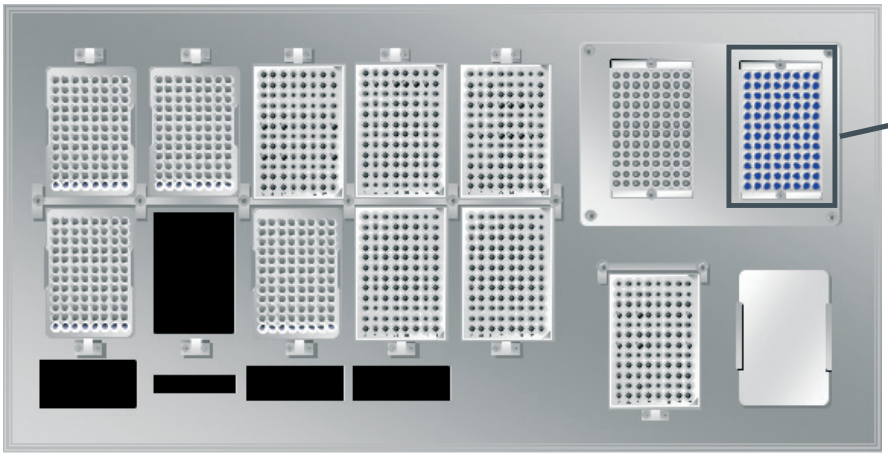
MagMeDIP protocols provided for the SX-8G IP-Star

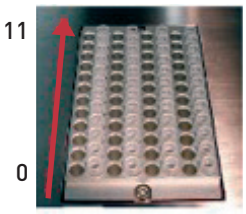
	Volumes	DIB	IPure
8 IP's	100 µl	☑	☑
16 IP's	100 µl	☑	☑

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.

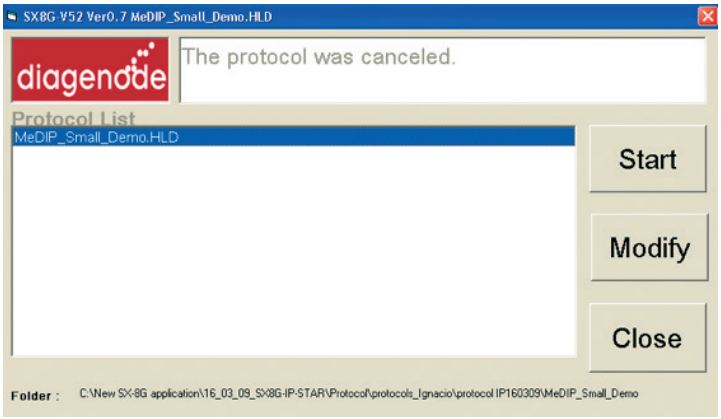
1. Switch on the SX-8G IP Star. The power switch is on the right side of the instrument.
2. Switch on the computer.
3. Start SX-8G V52 software through SX-8G V52 the following icon 
4. Place the prepared tube strip on the right cooling / heating block of the workstation





5. Close the workstation door and lock it using the following icon 
6. Press the following icon 

Select the protocol of interest. Press start.



IMPORTANT NOTE:

1. If the MeDIP protocols do not appear on the screen, Open the SX-8V52 directory and open **Easy start ini file. Write the directory location of the protocols**.

2. The **Easy start ini file should contain the following information**:

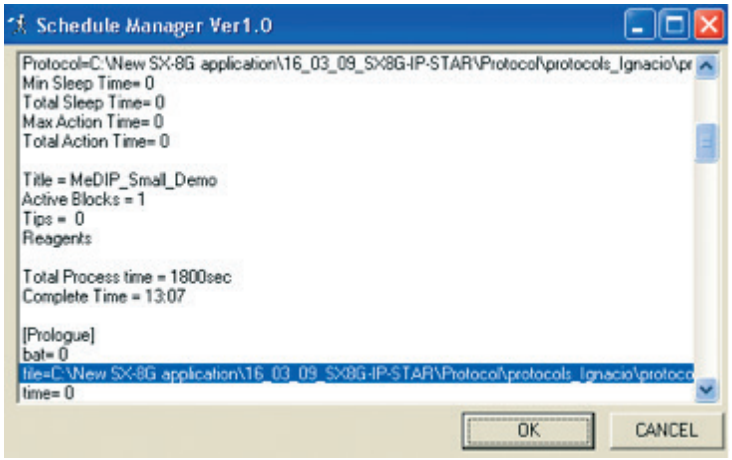
   [EASYSTARTSCREEN]

   HoldFilePath=**C:\Documents and Settings\Desktop\New software protocols\ MeDIP**

   In red is indicated the the directory location of the MeDIP protocols

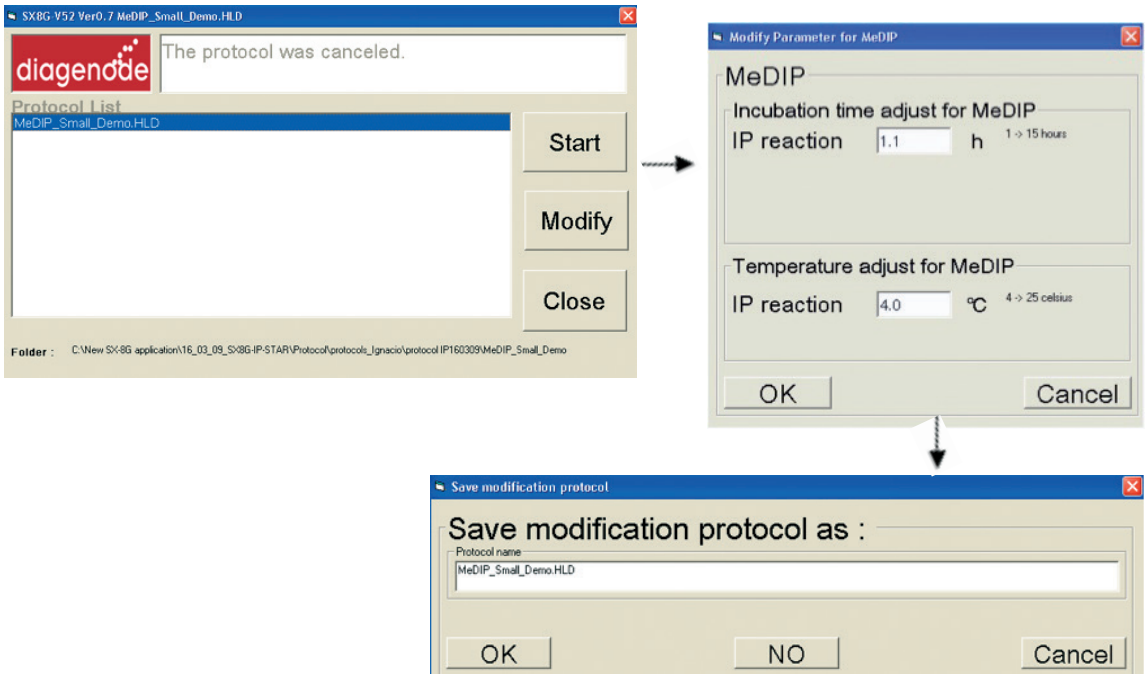
3. Start now SX-8G V52 software through **SX-8G V52 exe.file**

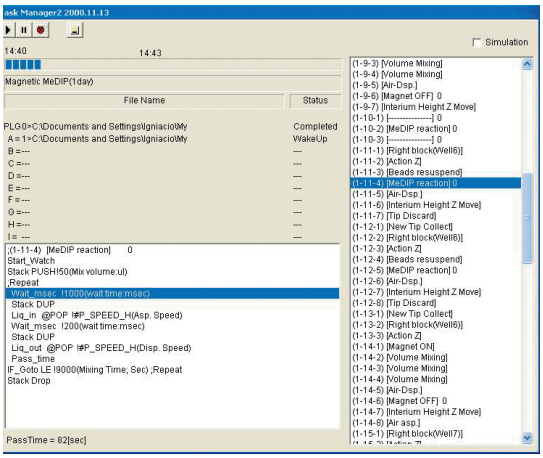
4. Press button for Easy Protocol Start screen and load the protocol of interest



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

Alternatively, temperature and incubation time for the IP reaction can be modified in an existing protocol by selecting the modify button. The modified protocol can be also saved as new protocol.



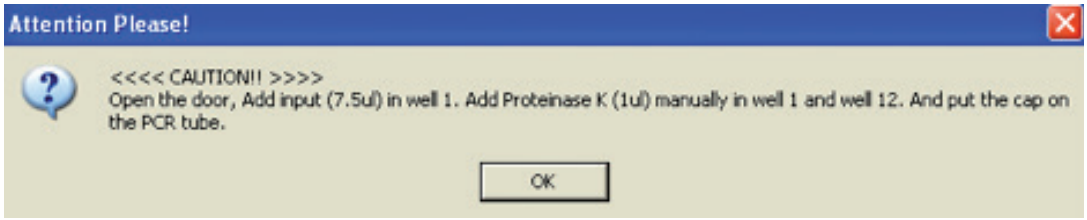


7. The program will run through the following steps: magnetic bead washes, IP and IP washes.

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

8. a) MagMeDIP-DIB

After the IP washes the following window will be appear.



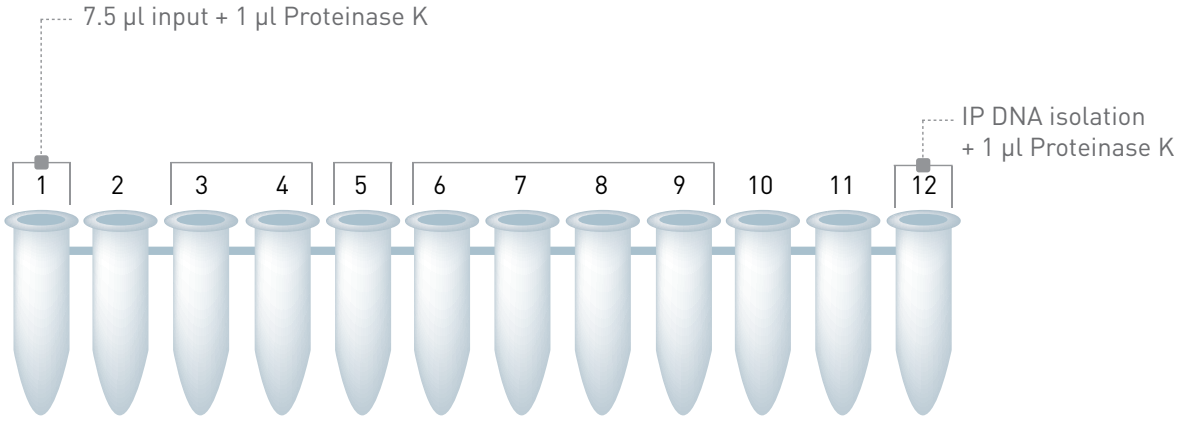
Follow the next instructions:

1. Add 7.5 µl of Incubation Mix (Input) to well 1

2. Add 1 µl proteinase K to wells 1 and 12

3. Close the tube strip with the corresponding caps

4. Press OK



IMPORTANT: Please note that the enriched methylated in DIB buffer is single strand DNA that can be directly analyzed by qPCR.

For downstream applications such as sequencing or arrays, the enriched methyated DNA needs to be purified by phenol/chroloform extraction and converted to double stranded DNA.

8. b) MagMeDIP-IPure

Follow the next instructions:

1. Collect samples from well 4 and keep them at 4 degrees
2. Follow instructions from the Auto IPure kit manual to proceed with the DNA purification of the samples and the input

IMPORTANT: Please note that the enriched methylated in DIB buffer is single strand DNA that can be directly analyzed by qPCR.

For downstream applications such as sequencing or arrays, the enriched methyated DNA needs to be purified by phenol/chroloform extraction and converted to double stranded DNA.

9. The following window will appear:

Close the workstation door and press OK.

The program will move forward to the next steps of the MeDIP protocol.

10. The SX-8G IP-Star software indicates the end of the protocol.

Collect your immunoprecipitated and isolated DNA

11. Discard magnetic beads by using the DiaMag02 (cat# kch-816-001) or by centrifugation.

12. This is your DNA ready for qPCR.

Shutting down the SX-8G 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 SX-8G IP-Star Automated System (power switch on the right side).

Note: Ensure that the door is closed!

Quantitative PCR & Data Analysis

The Methylated DNA IP module includes four validated primer pairs specific to four types of DNA:

1) methylated DNA control (primer pair #1)
2) unmethylated DNA control (primer pair #2).
3) methylated human DNA region (testis-specific H2B, TSH2B)
4) unmethylated human DNA region (GAPDH promoter)

Note: Primer pairs for mouse and rat are available! Please visit www.diagenode.com

1. Prepare your **qPCR mix** using SYBR Green PCR master mix and start out qPCR.

   qPCR mix (total volume of 25 µl/reaction):

   - 1.00 µl of provided primer pair (stock: 10 µM each: reverse and forward)
   - 12.50 µl of master mix (e.g.: iQ SYBR Green supermix)
   - 5.00 µl of isolated DNA or diluted purified DNA sample (see above for DNA dilutions)
   - 6.50 µl of water

Table 1. qPCR cycles:

	Temperature	Time	Cycles
PCR Amplification	95°C	7 minutes	x1
	95°C	15 seconds	x40
	60°C	60 seconds	
	95°C	1 minute	x1
Melting curve	65°C and increment of 0.5°C per cycle	1 minute	x60

2. When the PCR is done, analyse the results. Some major advices are given below.

   • **Data interpretation**

   The efficiency of methyl DNA immunoprecipitation of particular genomic locus can be calculated from qPCR data and reported as a recovery of starting material: % (meDNA-IP/ Total input).

   % (meDNA-IP/ Total input)= 2^[(Ct(10%input) - 3.32) - Ct(meDNA-IP)]x 100%

   Here 2 is the AE (amplification efficiency), Ct (meDNA-IP) and Ct (10%input) are threshold values obtained from exponential phase of qPCR for the methyl DNA sample and input sample respectively; the compensatory factor (3.32) is used to take into account the dilution 1:10 of the input. The recovery is the % (meDNA-IP/ Total input).

   • **Background determination**

   The final goal of IP is to calculate the enrichment in the same IP sample of: 1/ the specific DNA fragments (corresponding to the hydroxymethylated DNA) in comparison with 2/ non-methylated DNA (i.e. negative unDNA control).

   • **Relative occupancy can be calculated as a ratio of specific signal over background.**

   Occupancy= % input (specific loci) / % input (background loci)

   Relative occupancy is then used as a measure of the hydroxymethylation of a specific locus; it provides clues about specificity of the IP. (background loci) corresponds to the signal obtained with one of the unmethylated DNA kit control.

Results

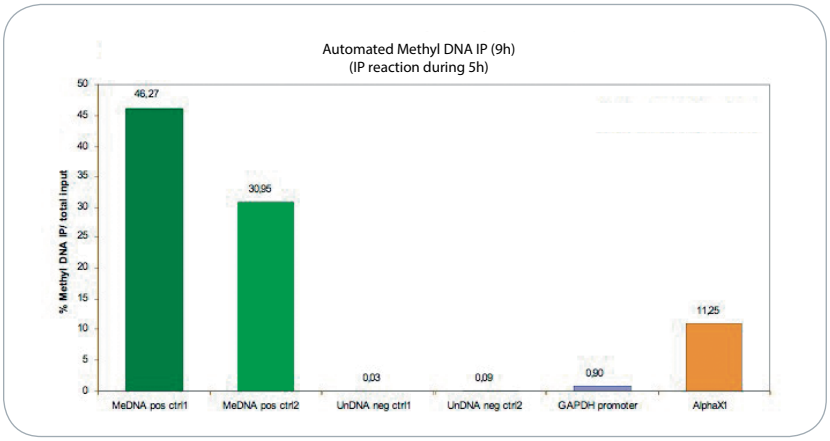


Figure 1: Automated MeDIP (9h)

IP reaction was performed with the anti-5meC antibody. Methylated and unmethylated DNA were used as internal controls. Unmethylated DNA region of GADPH and a methylated DNA region of AlphaX1 were used to test DNA sample-IP efficiency. DNA has been isolated by using DNA isolation buffer.

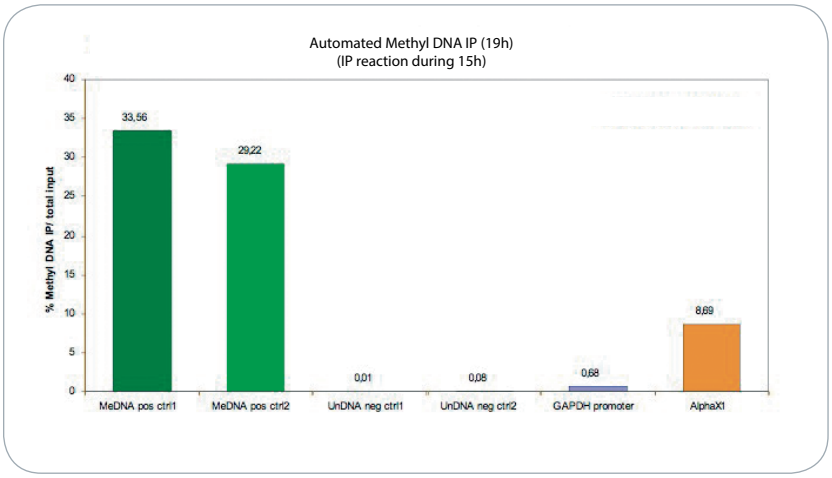


Figure 2: Automated MeDIP (19h)

IP reaction was performed with the anti-5meC antibody. Methylated and unmethylated DNA were used as internal controls. Unmethylated DNA region of GADPH and a methylated DNA region of AlphaX1 were used to test DNA sample-IP efficiency. DNA has been isolated by using DNA isolation buffer.

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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