

## **Auto Premium Bisulfite kit**

**Cat. No.** C02030031

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## Introduction

- Fastest method for complete bisulfite conversion of DNA for methylation analysis.
- Ready-to-use conversion reagent is added directly to DNA.
- High-yield, converted DNA is ideal for PCR, MSP, array, bisulfite and Next-Gen Sequencing.

## Product Contents

Description	Quantity	Storage
Conversion Reagent*	5 tubes	Room Temperature
Binding buffer	30 ml	Room Temperature
Wash buffer**	6 ml	Room Temperature
Desulphonation buffer	10 ml	Room Temperature
Elution buffer	1 ml	Room Temperature
Binding Beads	500 µl	Room Temperature
Instruction Manual	1	-

**Note** - Integrity of kit components is guaranteed for one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

\* The **Conversion reagent** is in a ready-to-use liquid format. The reagent should be stored tightly capped at room temperature with minimum exposure to light.

\*\* Add 24 ml of 100% ethanol to the 6 ml **Wash buffer** concentrate (K07291001) before use.

### Plastics and consumables available separately

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

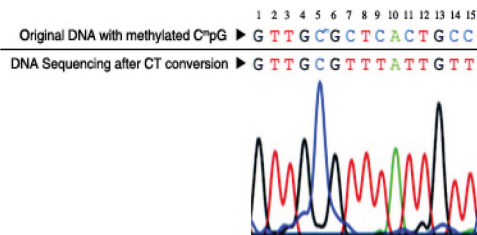
## Introduction to DNA Methylation

Cytosine methylation is a naturally occurring base modification, in both prokaryotic and eukaryotic organisms, consisting of the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring via a methyltransferase enzyme [1]. In prokaryotes DNA methylation provides a way to protect host DNA from digestion by restriction endonucleases that are designed to eliminate foreign DNA. DNA methylation in higher eukaryotes functions in the regulation/control of gene expression [2].

The majority of DNA methylation in mammals occurs in 5D-CpG-3D dinucleotides, although other patterns do exist. About 80 percent of all 5D-CpG-3D dinucleotides in mammalian genomes are found to be methylated, and the majority of the twenty percent that remain unmethylated are within promoters or in the first exons of genes. It has been demonstrated that aberrant DNA methylation is a widespread phenomenon in cancer and may be among the earliest changes to occur during oncogenesis [3]. DNA methylation has also been shown to play a central role in gene imprinting, embryonic development, X-chromosome gene silencing, and cell cycle regulation.

The ability to detect and quantify DNA methylation efficiently and accurately has become essential for the study of cancer, gene expression, genetic diseases, and many other important aspects of biology. To date, a number of methods have been developed to detect/quantify DNA methylation including: high-performance capillary electrophoresis [4] and methylation-sensitive arbitrarily primed PCR [5]. However, the most common techniques used today still rely on bisulfite conversion [6].

Treating DNA with bisulfite chemically modifies non-methylated cytosines into uracil, methylated cytosines remain unchanged. Once converted, the methylation profile of the DNA can be determined using the desired downstream application. For single locus analysis, the region of interest is generally amplified following bisulfite conversion (i.e., bisulfite PCR) and then sequenced or processed for Pyrosequencing®. Recent advances in methylation detection also allow the investigation of genome-wide methylation patterns using technologies including array-based methods, reduced representation bisulfite sequencing (RRBS), and whole genome bisulfite sequencing [7].



**Figure 2: DNA sequencing results following bisulfite treatment.**

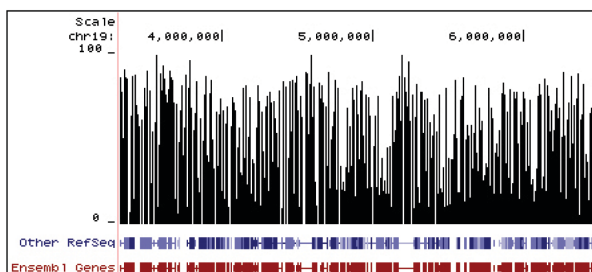
DNA with methylated C at nucleotide position #5 was processed using the Premium Bisulfite kit. The recovered DNA was amplified by PCR and then sequenced directly. The methylated cytosine at position #5 remains intact while the unmethylated cytosines at positions #7, 9, 11, 14 and 15 are completely converted into uracil following bisulfite treatment (detected as thymine following PCR).

### References

1. Adams RL. Bioessays. 1995; 17[2]: 139-145.
2. Costello JF, Plass CJ. Med. Genet. 2001; 38[5]: 285-303.
3. Stirzaker C. Cancer Res. 1997; 57[11]: 2229-2237.
4. Fraga MF, et al. Electrophoresis. 2000; 21[14]: 2990-2994.
5. Gonzalgo ML. Cancer Res. 1997; 57[4]: 594-599.
6. Frommer M. Proc. Natl. Acad. Sci. USA. 1992; 89[5]: 1827-1831.
7. Rakyan VK, et al. Nat. Rev. 2011; 12[8]: 529-541.

## Product Description

The Premium Bisulfite kit features rapid and reliable bisulfite treatment and conversion of DNA for methylation analysis. Key to the fast workflow is the ready-to-use Conversion reagent. No preparation is necessary, simply add this unique reagent to a DNA sample, wait about an hour, and let the reaction proceed to completion. DNA denaturation and bisulfite conversion processes are combined with added heat to facilitate rapid denaturation. Desulphonation and clean-up of the converted DNA is performed using a unique low-elution spin column. High yield, converted DNA is ideal for PCR, array, bisulfite and Next-Generation Sequencing, etc.



**Figure 2: Methylation Plot From Reduced Representation Bisulfite Sequencing (RRBS).**

Data shows the relative percentage of methylation at individual CpG sites in mouse DNA. Methylation percentage is shown across a ~3 Mb region of mouse chromosome 19. Bisulfite sequencing libraries were prepared using mouse genomic DNA prepped with the Genomic Clean & Concentrator™ (D4010, D4011 – Zymo Research) and bisulfite converted using Premium Bisulfite kit technology prior to Next-Gen Sequencing.

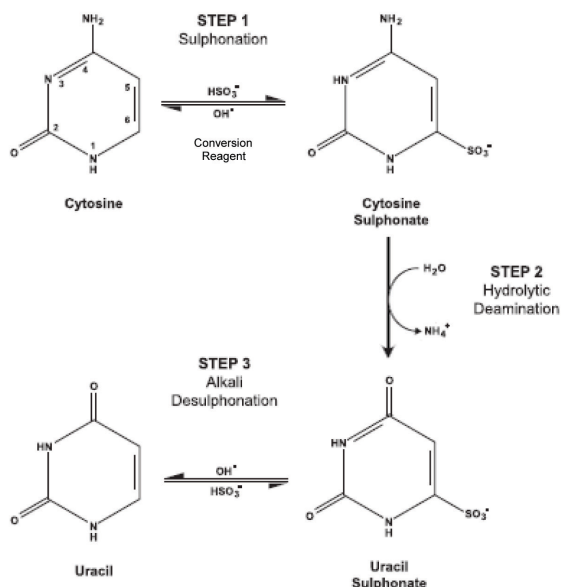
## Specifications

- DNA Input: Samples containing between 100 pg to 2 µg of DNA. For optimal results, the amount of input DNA should be from 200 to 500 ng.
- Conversion Efficiency: > 99.5% of non-methylated C residues are converted to U; > 99.5% protection of methylated cytosines.
- DNA Recovery: >80%

## Reagent Preparation

- Preparation of Wash buffer

Add 24 ml of 100% ethanol to the 6 ml Wash buffer concentrate (K07291001) before use.



**Figure 4: Overview of Bisulfite Conversion.**

Steps 1 and 2 occur during bisulfite conversion, while Step 3 is performed as the DNA is bound to the column matrix. For the reaction to proceed to completion, it is essential the DNA be fully denatured.

## SX-8G IP-Star® Compact



SX-8G IP-STAR® COMPACT

# How to perform Automated Bisulfite Conversion in the SX-8G IP-Star® Compact

## Protocol

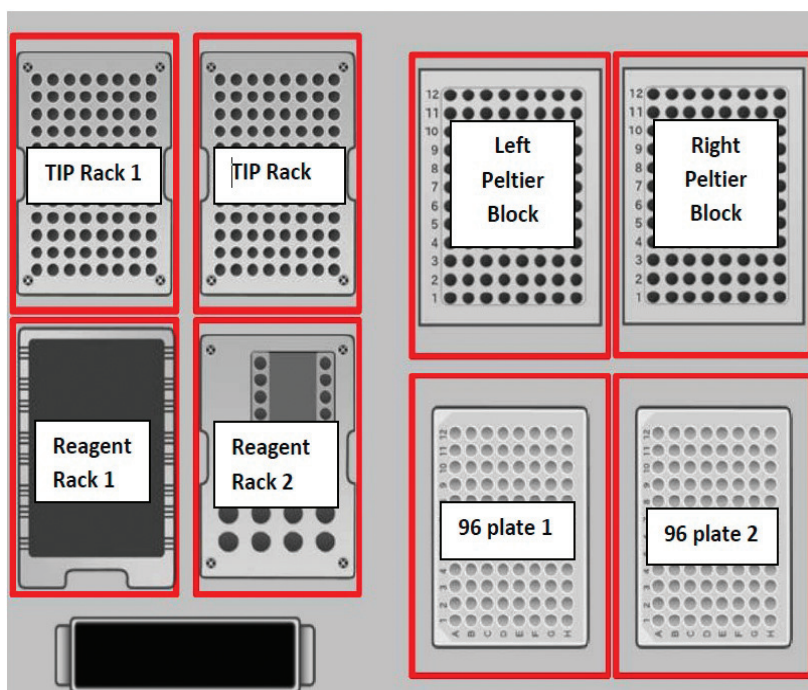
1. Add **46 µl of Conversion Reagent** to **5.4 µl of a DNA sample** in a PCR 200 µl tube strips (C30020002, WA-002-0120). Mix the samples by pipetting up and down and centrifuge briefly to ensure there are no droplets in the cap or sides of the tube.
2. Put the caps on the PCR tube strips. Transfer the PCR tube strips to a thermal cycler and perform the following steps:
  1. 98°C for 8 minutes
  2. 54°C for 60 minutes
  3. 4°C storage for up to 20 hours

**Note:** The 4°C storage step is optional.

3. Switch on the IP-Star®
4. Select “**Protocols**” icon and then “**MagBisulfite**”
5. Under “**MagBisulfite**”, select “**Premium\_Bisulfite**”
 

**Note:** If you plan to run between 1 and 8 samples, chose “**Premium\_Bisulfite\_08**”  
If you plan to run between 9 and 16 samples, chose “**Premium\_Bisulfite\_16**”
6. Setup the exact number of samples
 

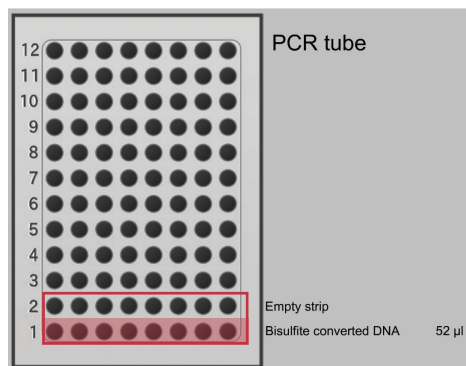
**Note:** The Left Peltier Block is now cooling down to 4°C to keep your samples cold
7. Setup all the plastics according to the screen layout.



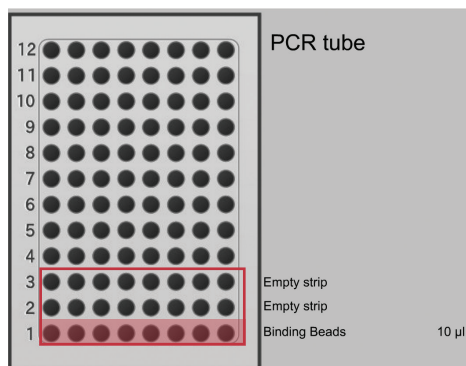
- Fill TIP Rack 1 (and 2 if processing 16 protocol) with tips according to the screen.
- Fill Reagent Rack 1 & 2 with reagent containers according to the screen.
- Fill 96 plate 1 with 96 well microplates.
- Fill Left Peltier Blocks with 200 µl tube strips according to the screen.
- Fill Right Peltier Block with 200 µl tube strips according to the screen.
- Fill the Left Peltier Block with the samples according to the screen layout.



8. Put the tubes containing **52 µl of converted DNA** on the Left Block



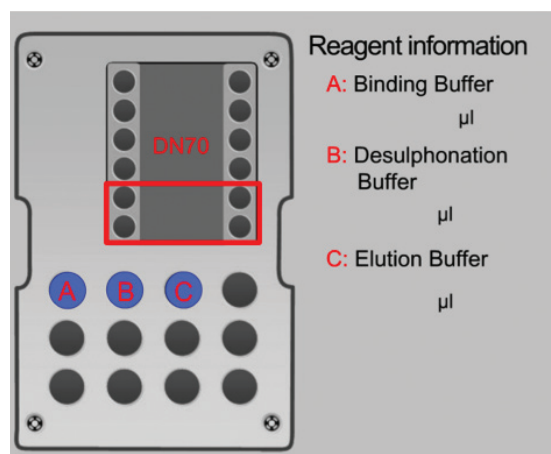
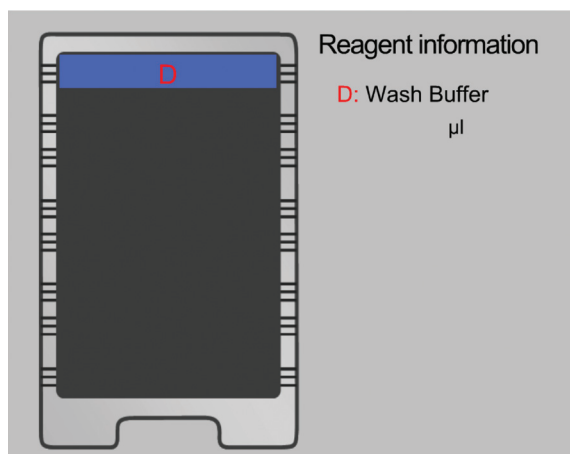
9. Setup **10 µl of Binding Beads** to the Right Block.



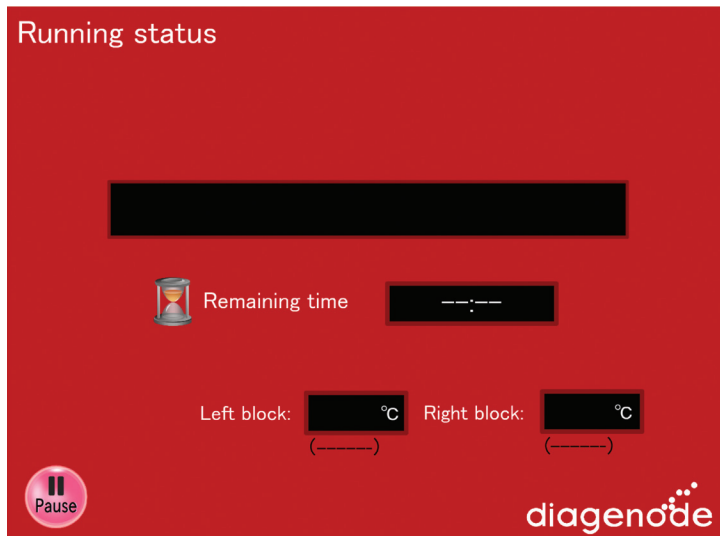
**Note:** Binding Beads settle very quickly, ensure that beads are kept suspended in the reservoir while adding to the tubes.

10. Setup the rest of the reagents

- Fill **Wash Buffer** in the container on **Reagent Rack 1**.
  - Fill **Binding Buffer** in the container on the **Reagent Rack 2**.
  - Fill **Desulphonation Buffer** in the container on the **Reagent Rack 2**.
  - Fill **Elution Buffer** in the container on the **Reagent Rack 2**.
- Note:** Alternatively, water or TE (pH ≥ 6.0) can be used for elution if required for your experiments.
- Close the door and Run.

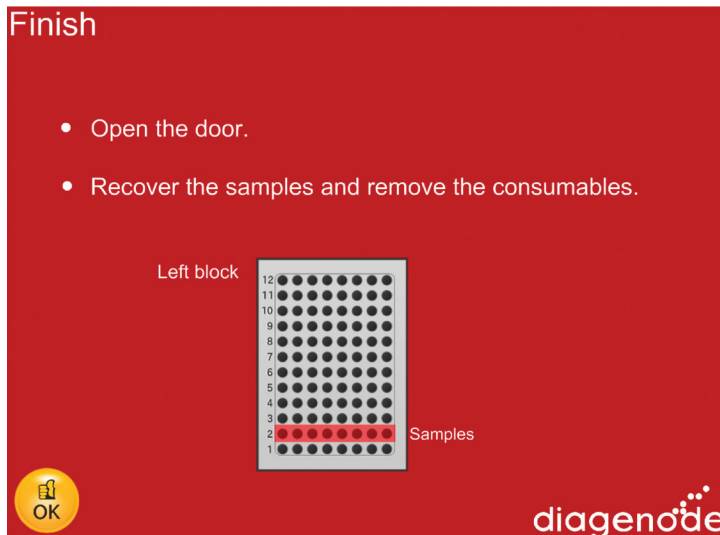


## 11. Protocol running



## 12. End: Recover your samples

The DNA is ready for immediate analysis or can be stored at or below -20°C for later use. For long term storage, store at or below -70°C. We recommend using 1-4 µl of eluted DNA for each PCR, however, up to 10 µl can be used if necessary. The elution volume can be > 10 µl depending on the requirements of your experiments, but small elution volumes will yield higher DNA concentrations.



## SX-8G IP-Star®




SX-8G IP-STAR®

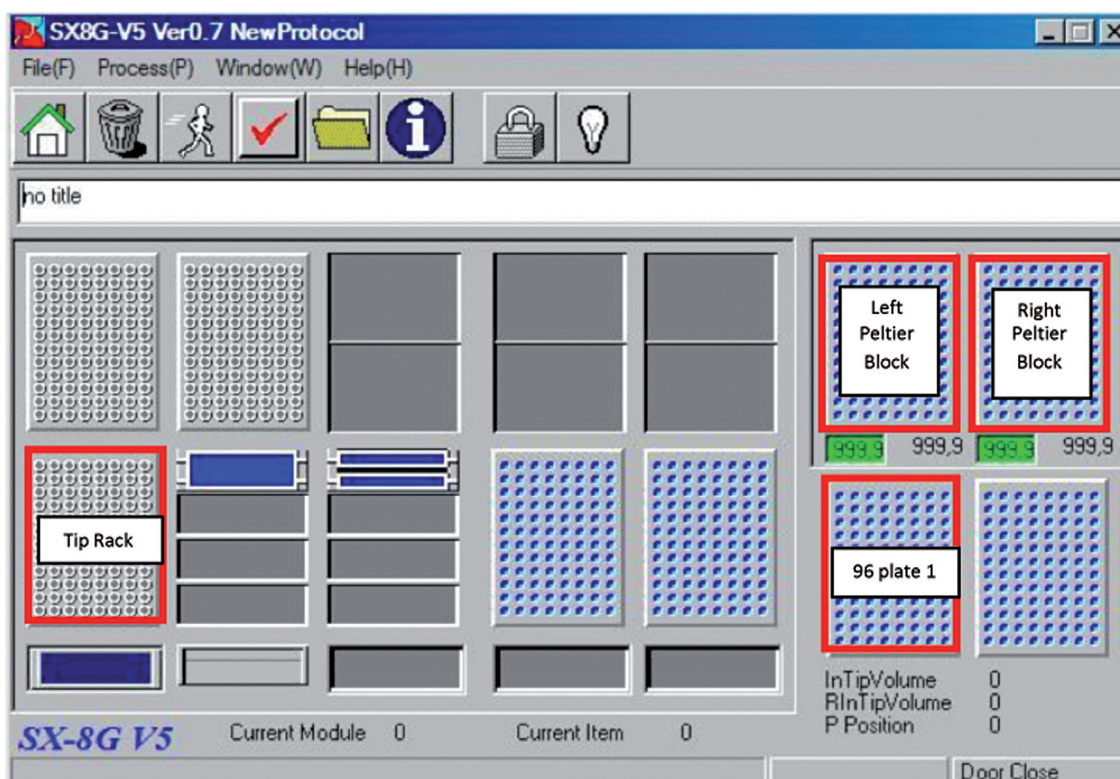
# How to perform Automated Bisulfite Conversion in the SX-8G IP-Star®

## Protocol

1. Add **46 µl of Conversion Reagent** to **5.4 µl of a DNA sample** in a PCR 200 µl tube strips (C30020002, WA-002-0120). Mix the samples by pipetting up and down and centrifuge briefly to ensure there are no droplets in the cap or sides of the tube.
2. Put the caps on the PCR tube strips. Transfer the PCR tube strips to a thermal cycler and perform the following steps:
  1. 98°C for 8 minutes
  2. 54°C for 60 minutes
  3. 4°C storage for up to 20 hours

**Note:** The 4°C storage step is optional.

3. Switch on the SX-8G IP Star. The power switch is on the right side of the instrument
4. Switch on the computer.
5. Start SX-8G V52 software through the following icon 
6. Setup the Temperature of the Left Peltier Block to 4°C  
Window(W) → Commander(C) → Set Temp Left → Arg2: 40 → Execute
7. Fill the Tip Rack with tips



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



Left Peltier Block	
12	
11	
10	
9	
8	
7	
6	
5	
4	
3	
2	25 µl Elution Buffer
1	52 µl Converted DNA

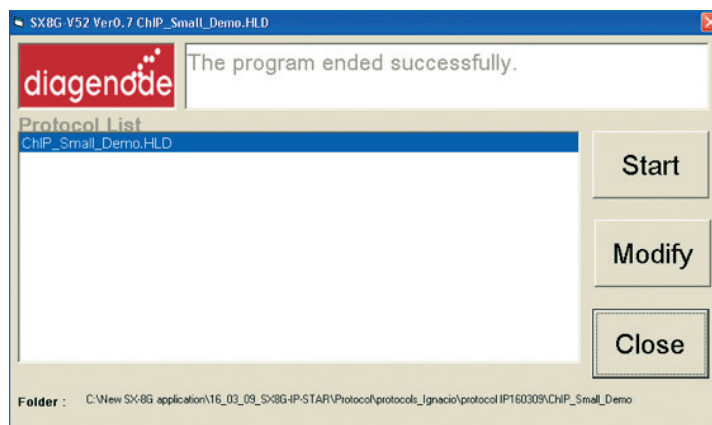
**Note:** Alternatively, water or TE (pH ≥ 6.0) can be used for elution if required for your experiments.

Right Peltier Block	
12	
11	
10	
9	
8	
7	
6	
5	
4	
3	200 µl Desulphonation Buffer
2	150 µl of Binding Buffer
1	10 µl of of Binding Beads

**Note:** Binding Beads settle very quickly, ensure that beads are kept suspended in the reservoir while adding to the tubes.

96 plate 1	
12	
11	
10	
9	
8	
7	
6	100 µl Wash Buffer
5	100 µl Wash Buffer
4	100 µl Wash Buffer
3	100 µl Wash Buffer
2	100 µl Wash Buffer
1	100 µl Wash Buffer

9. Close the workstation door and lock it using the following icon 
10. Press the following icon 
11. Select the protocol of interest "Premium\_Bisulfite\_08". Press star



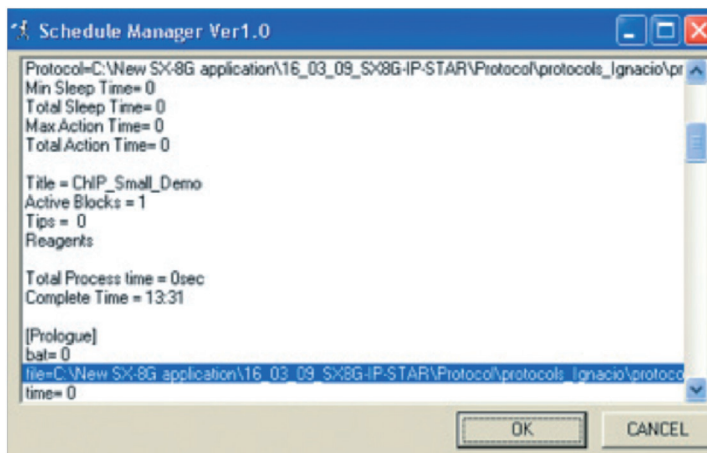
- Note:** If the **Premium Bisulfite** protocol do not appear in the screen,
1. Open the SX-8V52 directory
  2. Open Easy start ini file. Write the directory location of the protocols

The Easy start ini file should contain the following information:

[EASYSTARTSCREEN]

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

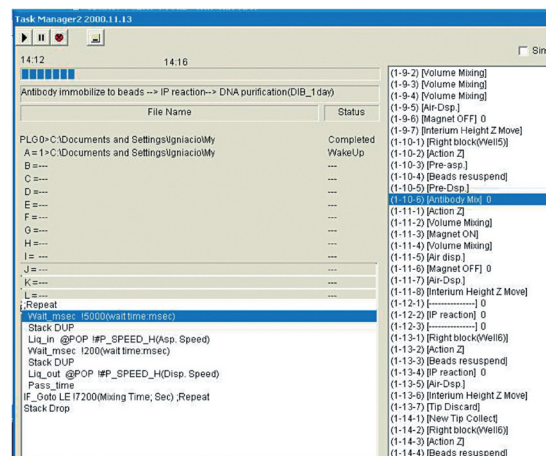
**Note:** Before starting the protocol a start confirmation window will appear. Press OK and the protocol will run.



## 12. Running Protocol

Be sure that the computer connected to the SX-8G IP-Star® never switches to the standby modus. (standby modus has to be inactivated). Standby of the computer will lead to the abort of the protocol.

Steps	Premium_Bisulfite_08
Conversion	75 min
Reagent preparation	15 min
Binding beads	5 min
Washes	30 min
Drying beads	30 min
Desulphonation	15 min
Elution	5 min



During protocol the next window will be displayed indicating the step that the protocol is processing

## 13. End: Recover your samples.

The DNA is ready for immediate analysis or can be stored at or below -20°C for later use. For long term storage, store at or below -70°C. We recommend using 1-4 µl of eluted DNA for each PCR, however, up to 10 µl can be used if necessary. The elution volume can be > 10 µl depending on the requirements of your experiments, but small elution volumes will yield higher DNA concentrations.

## 14. 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 SX-8G IP-Star® Automated System (power switch on the right side).

**Note:** Ensure that the door is closed!



## Appendix: Bisulfite Conversion and PCR Optimization

1. **Bisulfite Conversion of Double Stranded DNA Templates.** The following illustrates what occurs to a DNA template during bisulfite conversion.

Template:           **A:** 5'-GACCGTTCCAGGTCCAGCAGTGCGCT-3'  
                       **B:** 3'-CTGGCAAGGTCCAGGTCGTCACGCGA-5'

Bisulfite Converted: **A:** 5'-GATCGTTTTAGGTTTAGTAGTGCGTT-3'  
**B:** 3'-TTGGCAAGGTTTAGGTTGTTATGCGA-5'

**Note:** Methylated "C" is underlined in the examples.

**Note:** Following bisulfite conversion, the strands are no longer complementary.

- 2. PCR Primer Design.** Generally, primers 26 to 32 bases are required for amplification of bisulfite converted DNA. In general, all Cs should be treated as Ts for primer design purposes, unless they are in a CpG context. See example below.

Bisulfite Converted: A: 5'-GATCGTTTTAGGTTAGTAGTGCGTT-3'

Primers: Reverse: 3'-ATCATCAC**R**CAA-5' R= G/A

Forward: 5'-GAT**YG**TTTTAG**GG**T-3' Y= C/T

**Note:** Only one strand (A) is amplified by a given primer set. Only the reverse primer binds to the converted DNA, the forward primer will bind the strand generated by the reverse primer.

If the primer contains CpG dinucleotides with uncertain methylation status, then mixed bases with C and T (or G and A) can be used. Usually, there should be no more than one mixed position per primer and it should be located toward the 5' end of the primer. It is not recommended to have mixed bases located at the 3' end of the primer.

3. **Amount of DNA Required for Bisulfite Conversion.** The minimal amount of human or mouse genomic DNA required for bisulfite treatment and subsequent PCR amplification is 100 pg. The optimal amount of DNA per bisulfite treatment is 200 to 500 ng. Although, up to 2 µg of DNA can be processed, it should be noted that high input levels of DNA may result in incomplete bisulfite conversion for some GC-rich regions.
4. **PCR Conditions.** Usually, 35 to 40 cycles are required for successful PCR amplification of bisulfite converted DNA. Optimal amplicon size should be between 150-300 bp; however larger amplicons (up to 1 kb) can be generated by optimizing the PCR conditions. Annealing temperatures between 55-60°C typically work well. As most non-methylated cytosine residues are converted into uracil, the bisulfite-treated DNA usually is AT-rich and has low GC composition. Non-specific PCR amplification is relatively common with bisulfite treated DNA due to its AT-rich nature. PCR using "hot start" polymerases is strongly recommended for the amplification of bisulfite-treated DNA.
5. **Quantifying Bisulfite Treated DNA.** Following bisulfite treatment of genomic DNA, the original base-pairing no longer exists since non-methylated cytosine residues are converted into uracil. Recovered DNA is typically A, U, and T-rich and is single stranded with limited non-specific base-pairing at room temperature. The absorption coefficient at 260 nm resembles that of RNA. Use a value of 40 µg/ml for Ab260 = 1.0 when determining the concentration of the recovered bisulfite-treated DNA.

## Frequently Asked Questions

**Q: Should the input DNA be dissolved in TE, water, or some other buffer prior to its conversion?**

**A:** Water, TE or modified TE buffers can be used to dissolve the DNA and do not interfere with the conversion process.

Q: Which Taq polymerase(s) do you recommend for PCR amplification of converted DNA?

**A:** We recommend Diagenode's MethylTag DNA polymerase (Cat. No. C09010010).

## Ordering information

Description	Cat. No. (NEW)	Cat. No. (OLD)	Format
SX-8G IP-Star® Compact	B03000002	UH-002-0001	1 unit
Auto Premium Bisulfite kit	C02030031	/	40 rxns
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
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 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	C03010010	AL-Auto01-0100	100 rxns

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