diagenode
A Hologic Company

Automated ChIP with the SX-8G IP-Star®

Screening histone modifications in genomic regions using chromatin from 1,000 to 1 million cells

Data provided with the contribution of Ignacio Mazon Pelaez [1], Lorigail Echipare [2] and Peggy Farnham [2].

(1) Diagenode
(2) Department of Pharmacology, University of California Davis

Customer feedback

"We have found that the SX-8G IP-Star provides a highly reproducible and high throughput alternative to manual ChIP assay for the analysis of histone modifications. The automated system allows investigators to run more assays with greatly reduced "hands-on" time, freeing them for critical upstream procedures and downstream analyses. Most importantly for the epigenomic analyses of primary cells and tissues, the IP-Star provides robust ChIP enrichments of targets using very small numbers of cells."

Dr. Peggy Farnham, Department of Pharmacology, University of California Davis

diagenode
A Hologic Company

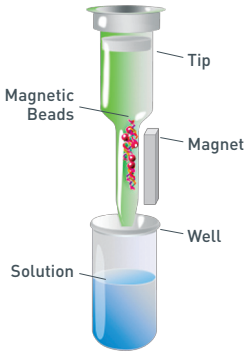
The Diagenode SX-8G IP-Star® Automated System automates immunoprecipitation and increases reproducibility

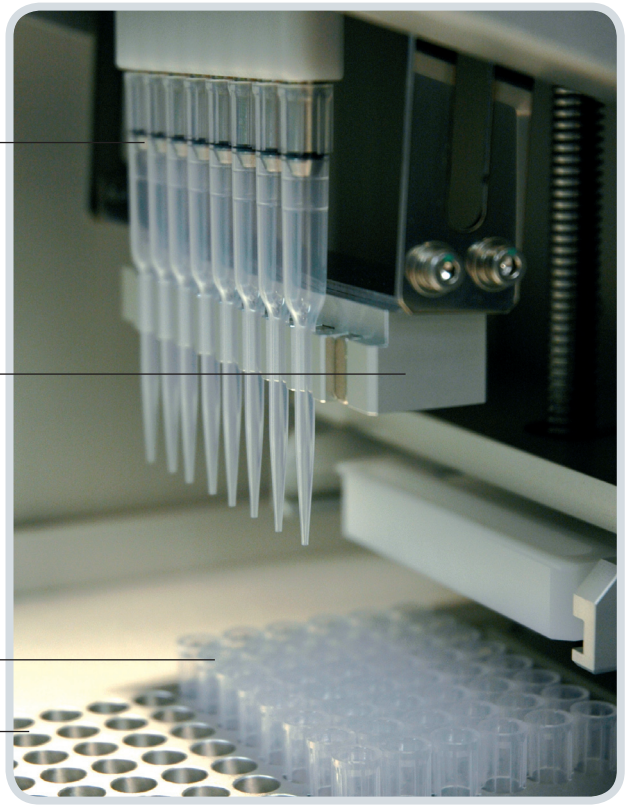
Diagenode, the leading provider of complete solutions for epigenetics research, offers a variety of end-to-end systems to streamline methylation and chromatin immunoprecipitation workflows. Central to this full offering is Diagenode's SX-8G IP-Star Automated System (the "IP-Star"), a simple yet robust bench-top instrument that standardizes Chromatin Immunoprecipitation and DNA methylation assays. We specifically designed the IP-Star to make ChIP and methylation studies accessible and reproducible, to deliver consistent data in every study. The IP-Star will produce consistent results from any operator regardless of the day, the experimental run, or the lab, a major goal of today's high resolution and genome-wide epigenetics studies.

The IP-Star replaces the numerous manual, error-prone steps of immunoprecipitation with a reliable, highly-consistent and automated process that requires minimal operator intervention. The IP-Star lab "workhorse" allows researchers to achieve higher data reproducibility, more confidence in their results, increased efficiency, greater throughput, and the flexibility to reallocate personnel to more productive tasks. With our automated IP-Star workstation, we empower researchers to simplify the tedious protocols and the complexity associated with manual ChIP and DNA methylation workflows. In addition, the IP-Star minimizes sample carryover, data variability, and costly errors. The IP-Star platform offers full workflow support for epigenetics research, by being fully-compatible with our complete kits and laboratory-validated protocols to rapidly deliver high-quality and consistent data.

Figure 1
Overview of the SX-8G IP-Star® Automated System

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.



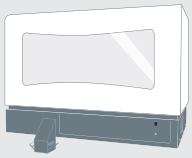
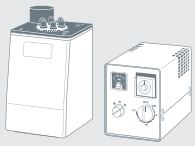
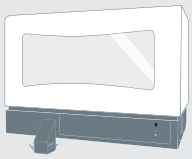
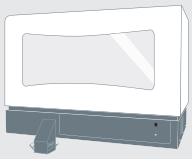
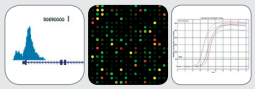


diagenode
A Hologic Company

Applications on the SX-8G IP-Star®

The SX-8G IP-Star Automated System has been validated across diverse methods for optimized chromatin immunoprecipitation and DNA methylation studies. Our ChIP and DNA methylation kits provide the versatility, convenience, and reproducibility required for all your epigenetics research. For ChIP assays on the SX-8G IP-Star, we offer the Auto ChIP Kit for unparalleled reproducibility starting with as few as 1,000 cells. For DNA methylation assays with the SX-8G IP-Star, we offer optimized kits for both methylated DNA immunoprecipitation (MeDIP) and Methylation Binding Domain Protein Affinity Separation (MBD). Diagenode also offers automated protocols for IPure, the only DNA purification method that is specifically optimized for extracting very low amounts of DNA after ChIP and MeDIP assays. Furthermore, the same technology can be used for sample preparation e.g. DNA or RNA extraction (available soon).

Figure 2. Automated assay and sample processing workflows for ChIP and methylation studies

	PROTOCOLS	REAGENTS
Sample Preparation 	☑ DNA from whole blood ☑ Total nucleic acid purification from cultured cells ☑ DNA Purification of PCR amplicon ☑ DNA purification from cultured cells ☑ RNA purification from cultured cells ☑ C → U DNA clean up from bisulphite solution	Available soon
Shearing 	☑ DNA shearing protocol ☑ Chromatin shearing protocol	☑ Shearing ChIP kit ☑ Shearing Optimization kit
Immunoprecipitation 	☑ MeDIP 8 IP's ☑ MeDIP 16 IP's ☑ ChIP 8 IP's ☑ ChIP 16 IP's ☑ MethylCap One Elution step ☑ MethylCap Fractionated Elution	☑ Auto ChIP kit ☑ Auto MeDIP kit ☑ Auto MethylCap kit ☑ ChIP & MeDIP grade antibodies
DNA Purification 	☑ DNA Isolation Buffer ☑ IPure	☑ IPure kit
Downstream Application 	☑ qPCR ☑ Arrays ☑ Next Generation Sequencing	

diagenode
A Hologic Company

Automated ChIP assays with the SX-8G IP-Star®

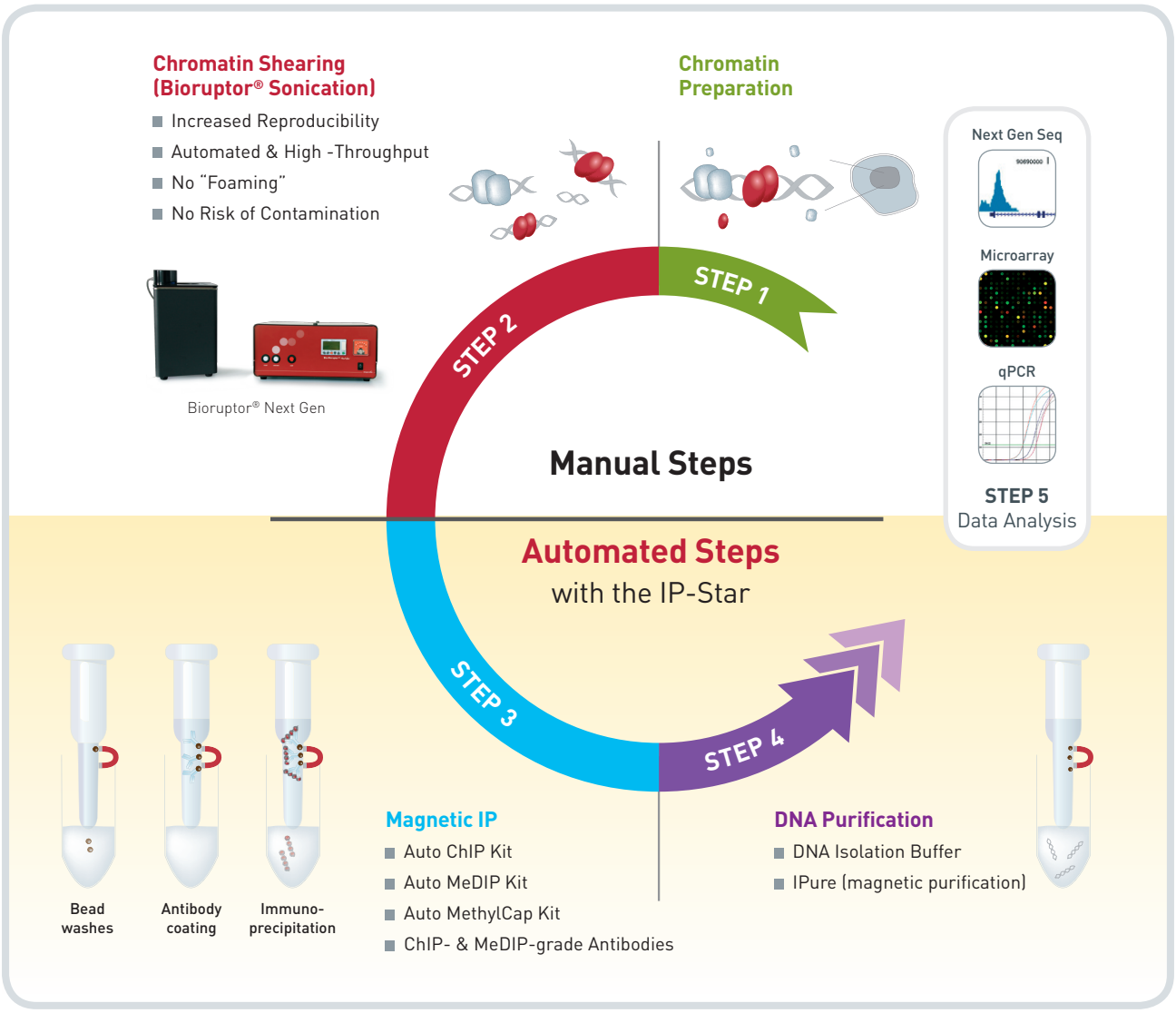


Figure 3. Diagenode provides a full product offering for ChIP chromatin preparation kits. In step 1, we offer to isolate nuclei and chromatin. Step 2 describes reproducible sample shearing with the Bioruptor® product line. In Step 3 and 4, the Diagenode IP-Star provides error-free, walk-away automation for all your immunoprecipitation and antibody capture needs.

Customer feedback

Our results are much less influenced by random variation if the immunoprecipitation is performed with the SX-8G IP-Star System. For most genes, similar methylation profiles are obtained but [are] much less 'noisy'. In some cases these differences might lead to the detection of additional differentially methylated genes.

Jörg Tost, Centre National de Génotypage, IP-Star customer

Results

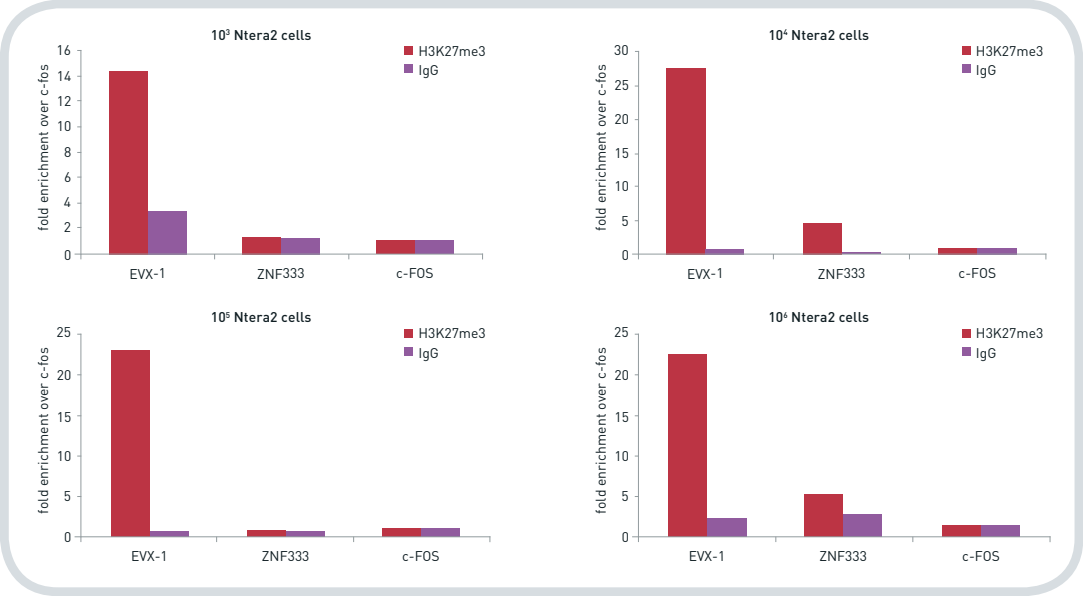


Figure 4. Results for Auto ChIP are shown using the H3K27me3 antibody with chromatin from 10^3 to 10^6 cells. Results are graphed as fold enrichment over a negative locus, c-fos. EVX-1 was tested as positive control locus whereas the promoter regions of ZNF333 and c-fos promoter region were used as negative control loci. ChIP assays were performed in the SX-8G IP-Star automated system. H3K27me3 antibody amounts were the following: for chromatin from 10^6 cells, 2 µg of H3K27me3; for 10^5 and 10^4 cells 1 µg of H3K27me3; and for 10^3 cells; 0.5 µg of H3K27me3.

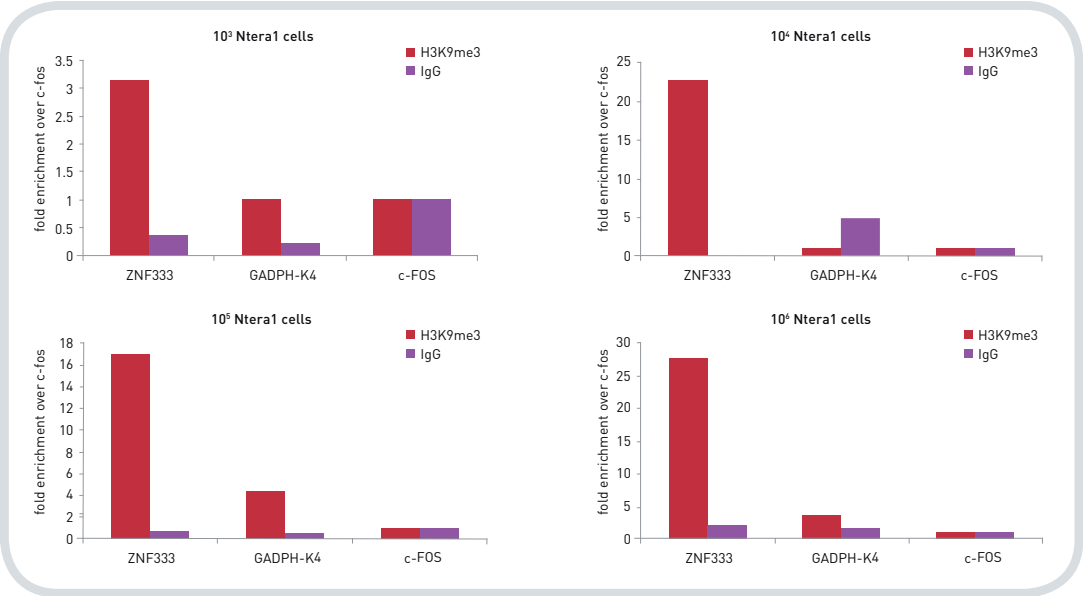


Figure 5. Results for Auto ChIP are shown with H3K9me3 antibody with chromatin from 10^3 to 10^6 cells. Results are graphed as fold enrichment over a negative genomic locus,c-fos. ZNF333 was tested as positive control locus whereas the promoter regions of GADPH-K4 and c-fos promoter region were used as negative control loci. ChIP assays were performed in the SX-8G IP-Star automated system. H3K9me3 antibody amounts were the following: for chromatin from 10^6 cells, 2 µg of H3K9me3; for 10^5 and 10^4 cells 1 µg of H3K9me3; and for 10^3 cells 0.5 µg of H3K9me3.

Automated ChIP protocol

Auto ChIP kit

With the Auto ChIP kit, the protocol was developed to enhance the utility of the ChIP procedure, allowing one to perform many more ChIPs per day and per week. The entire procedure can be performed in a single day, given that the two overnight incubations have been eliminated. The IP has been optimized to specifically select and precipitate the chromatin with the use of our validated antibodies, buffers and protocols. Furthermore, the use of our automated system will drastically increase the consistency of your ChIP assay, a critical need based on manual ChIP results.

The Auto ChIP kit allows chromatin IP sample analysis in a fast, high and specific manner. In the Auto ChIP kit, the protocol has been improved to allow researchers to work in smaller tubes than traditionally used. The kit ensures the use of small amounts of reagents per reaction (including antibodies and buffers) and also provides you with fewer buffers in comparison with other kits.

The Auto ChIP Kit also includes a DNA isolation buffer (DIB) 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 can be automated (IPure). The combination of this high quality kit and the IP-Star allows Chromatin IP to be performed in less than 10 hours. Starting with sheared chromatin, the Automated System provides purified immunoprecipitated DNA of your sample. The Auto ChIP kit protocol has been validated using chromatin sheared by sonication using the Bioruptor

Protocol

1. Ntera2 cells were cultured under appropiate growth conditions.
2. 10^8 cells were collected and nuclei was isolated.
3. Chromatin of 10^7 cells was sheared in 130 µl of Buffer B containing protease inhibitors (sheared chromatin from 10^7 cells in 200 µl of nucleic lysis buffer).
4. Bioruptor® (Cat No UCD-200-TM) was used for chromatin shearing. Sonication conditions were selected following Bioruptor® guidelines (conditions: pulses of 30 sec at setting high with 1.5 min pause in between pulses; total sonication time: 25 min).
5. After shearing, centrifugation at 1500 rpm was performed and the supernatant was transferred to a new tube.
6. 800 µl of Buffer A were added to the 200 µl of sheared chromatin.
7. 100 µl of the diluted sheared chromatin mix was used per reaction.
8. Antibody was added in the assigned well and Buffer A was added to bring the total volume to 100 µl.
   Antibody amounts were the following:
   For 10^6 cells: 2 µg histone antibody
   For 10^5 and 10^4 cells: 1 µg histone antibody
   For 10^3 cells: 0.5 µg histone antibody
   Antibodies tested: H3K27me3 and H3K9me3.
9. ChIP assay was performed in the SX-8G IP-Star.
10. Reverse crosslinking was done during 10 hours at 65°C.
11. RNAase treatment and DNA purification provided DNA ready for qPCR.
    Note: a combination of the Auto ChIP kit and Washing buffers from the Red ChIP kit were used to perform all the histone ChIP experiments.

Conclusions

"We have found that the SX-8G IP-Star provides a highly reproducible and high throughput alternative to manual ChIP assays for the analysis of histone modifications. The automated system allows investigators to run more assays with greatly reduced "hands-on" time, freeing them for critical upstream procedures and downstream analyses. Most importantly for the epigenomic analyses of primary cells and tissues, the IP-Star provides robust ChIP enrichments of targets using very small numbers of cells" (Dr. Peggy Farnham, Department of Pharmacology, University of California Davis).

SX-8G IP-Star® should be considered as your ideal labmate because:

- **Reproducibility:** Our standardized system and kits provide consistency between runs and users, increasing reproducibility of ChIP and DNA methylation results
- **Speed and accuracy:** Full walk-away automation saves time and eliminates tedious manual steps, minimizing cross-contamination, sample-carryover, and false positive rates
- **Sample efficiency:** Minimal sample loss and smaller reaction volumes allow scientists to use less starting material and eliminates the need for amplification, thus simplifying sample requirements
- **High-throughput capability:** SX-8G IP-Star® automation runs up to 16 samples in parallel in less than 10 hours; A second-shift of assays can be run overnight for 24 x 7 system performance
- **Flexibility and scalability:** SX-8G IP-Star® software provides an easy-to-use user interface, with open programming of protocols and throughput requirements to adapt protocols to your specific application
- **Ease of use:** Simple interface facilitates proper system set-up and error-free operation
- **Full workflow support:** SX-8G IP-Star® automation along with wide selection of epigenetics solutions including Bioruptor® Sonicator for optimal DNA shearing, optimized ChIP and DNA methylation kits, and DNA purification reagents assure data quality
- **Compatibility:** The system is compatible with popular downstream applications including qPCR, microarrays, and high-throughput ("next generation") sequencing

Additional information

Visit our website or contact us by e-mail to learn more about SX-8G IP-Star® and the related applications and products.

TN-IPS-V1-23_02_10