

# Comparison of Diagenode's LowCell# ChIP kit and commercially available kits

diagenode

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## Introduction

Determining the genomic targets of DNA-binding proteins is crucial in understanding transcriptional control, epigenetic silencing, gene regulation pathways, and cellular proliferation. Chromatin immunoprecipitation (ChIP) allows analysis of this association of proteins with specific genomic regions in vivo. To describe this method in brief, cells are first fixed with a reversible cross-linking agent followed by shearing of the cross-linked chromatin (the protein-bound DNA). Next, the DNA fragments associated with the protein of interest are immunoprecipitated (IP'd) using specific antibodies. Finally, the immunoprecipitated DNA is analyzed by qPCR, ChIP-chip, or sequencing for the enrichment of specific sequences associated with the protein of interest.

In recent years, several vendors have developed ChIP kits to overcome the tedious optimization ChIP typically requires. In this study, we compared four commercially available ChIP kits with the Diagenode LowCell# ChIP Kit (Cat. No. kch-maglow-A16). These kits include: ChIP-IT Express Kit (Active Motif), Magna ChIP A Kit (Millipore), Imprint Chromatin Immunoprecipitation Kit (Sigma), and MAGnify Chromatin Immunoprecipitation System Kit (Invitrogen). All ChIP assays were performed with ChIP-grade antibodies (Diagenode) directed against histone modifications (H3K9me3 and H3K27me3). The amount of chromatin used was based on each manufacturer's recommendations: 15 µg of chromatin for ChIP-IT Express and 100 000 cell equivalents for other kits. After ChIP, the IP'd DNA was analyzed by qPCR with primers specific for TSH2B as a positive locus and primers specific for the c-fos promoter as a negative locus.

## Results

### 1. Comparisons of ChIP kit efficiencies following each kit's procedures

Initially, we tested ChIP kits using the protocols provided by each manufacturer for all steps and with a validated ChIP-grade antibody. Chromatin used in each assay was prepared according to each kit's protocol. To test the efficiency of the different ChIP kits, we compared each kit to the Diagenode LowCell# ChIP Kit.

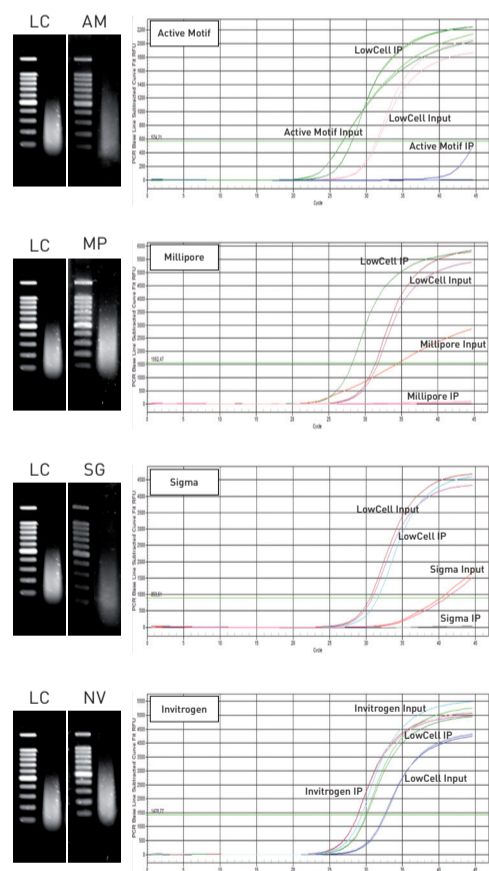


Figure 1. Pairwise kit comparisons using each kit's procedure for all steps. Upper Panel:

ChIP assays were performed using the LowCell# ChIP kit (LC) from Diagenode and: the Active Motif kit (AM), the Millipore kit (MP), the Sigma kit (SG) or the Invitrogen kit (NV). Chromatin from HeLa cells, the Diagenode antibody against H3K9me3 (Cat. No. pAb-056-050) and optimized primers for qPCR were used for all experiments. Left panels. Analysis of the DNA isolated from all chromatin batches prepared according to each kit's instructions and sheared using the Diagenode Bioruptor®. Right panels. PCR curves obtained after ChIP with H3K9me3 antibody (3 µg per reaction) using primers specific for TSH2B. E, F, G. ChIP results obtained with antibodies against H3K9me3 (3 µg/reaction) and H3K27me3 (2 µg/reaction) [Cat. No. pAb-069-050] using the Diagenode and commercial kit protocols. IgG was used as negative IP control. Figures E and F show the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis). The relative occupancy (calculated as a ratio of specific signal over background) is shown in Figure G.

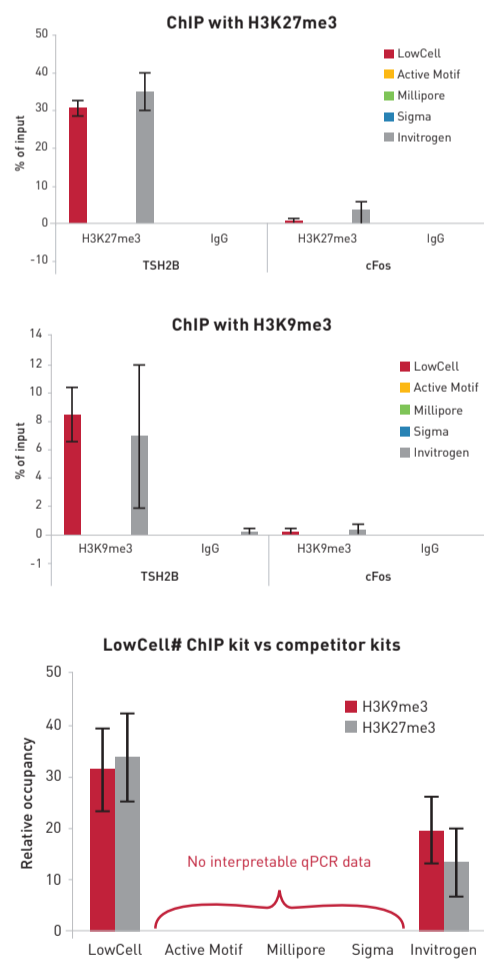


Figure 2. Comparison of ChIP kit efficiency using the Diagenode LowCell# ChIP kit Method for IP'd DNA purification.

ChIP experiments were performed with 3 µg of H3K9me3 antibody (Cat. No. pAb-056-050) or 2 µg of H3K27me3 antibody (Cat. No. pAb-069-050) according to each kit's instructions. The IP'd DNA was purified with DNA Isolation Buffer per the LowCell# ChIP Kit protocol. Next, qPCR was performed with primers for the TSH2B gene and for the c-fos promoter. Figure A shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis). The relative occupancy (calculated as a ratio of specific signal over background) is shown in Figure B.

### 2. ChIP efficiency using the LowCell# ChIP Kit purification method for IP'd DNA for each kit

We observed poor qPCR results from most of the commercial kits, possibly due to inefficiency in the purification methods of the IP'd DNA. In contrast, the Diagenode LowCell# ChIP Kit's purification method using the DNA Isolation Buffer gave efficient and specific results. Therefore, we performed additional ChIP experiments with the kits that gave poor results previously according to manufacturer recommendations but substituted the Diagenode method for IP'd DNA to allow for a more accurate comparison of kit efficiency.

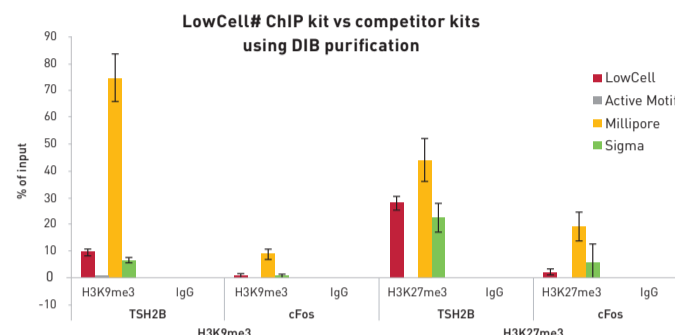


Figure 3. ChIP with the LowCell# ChIP kit on decreasing amount of cells.

To try to really challenge our LowCell# ChIP kit, we performed ChIP reaction on decreasing amount of chromatin. Serial dilutions of a same quantity of chromatin were used per ChIP reaction.

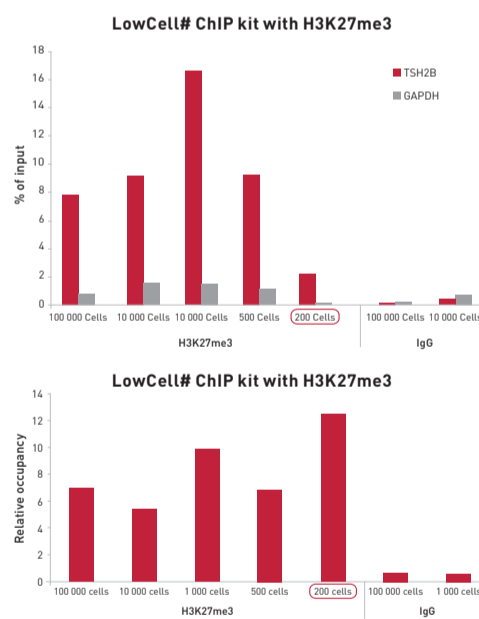


Figure 4. ChIP with the LowCell# ChIP kit on decreasing amount of cells.

ChIP assays were performed using the Diagenode LowCell# ChIP kit, HeLa cells, the Diagenode antibody directed against H3K27me3 (Cat. No. pAb-069-050) and the optimized primers for qRT-PCR using the SX-8G IP-Star Automated System. Chromatin was sheared from 1 million cells and serial dilutions of this chromatin (from 100 000 to 200 cell equivalent) were used per ChIP reaction. One µg of antibody and 10 µl of beads were used per ChIP experiment performed on 100 000 cell equivalent. ChIP on 100 000 and 1000 cell equivalent were performed using 0.5 µg of antibody and 10 µl of beads and 0.25 µg of antibody and 5 µl of beads were used for ChIP reaction with 500 and 200 cell equivalent. A negative control antibody was included in the ChIP assay (1 µg/IP with 100 000 cell equivalent and 0.5 µg/IP with 1000 cell equivalent). Figure A shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis). The relative occupancy (calculated as a ratio of specific signal over background) is shown in Figure B.

## Benefits of the Diagenode LowCell# ChIP Kit

- Highest specificity (signal-to-noise ratio)
- Fewer cells required for efficient ChIP
- Compatible with Automated System
- Easy to handle

## Conclusions

Summary of ChIP kit performance	Diagenode LowCell# ChIP kit	Active Motif ChIP-IT Express	Millipore Magna-ChIP	Sigma Imprint Chromatin Immunoprecipitation kit	Invitrogen MAGnify Chromatin Immunoprecipitation system
ChIP specificity	+++	-	-	-	+
Cell number/IP	200 - 1 000 000 cells	100 000 - 7 500 000	100 000 - 1 000 000 cells	100 000 - 250 000 cells	10 000 - 300 000 cells
Compatible with Automated system*	+++	-	-	-	-
DNA purification efficiency	+++	-	-	-	+
Handling	+++	+	+	+++	+++

\* SX-8G IP-Star Automated System