

Rapid Chromatin Immunoprecipitation from a low number of cells with LowCell# ChIP Kit

The LowCell# ChIP kit allows the identification by chromatin immunoprecipitation of genomic sequences bound to specific proteins from as few as 1,000 cells. Using the kit, we demonstrate the association of epigenetically modified histones and of a transcription factor to various genomic sites and address downstream applications of the kit. The data illustrate the high specificity and reproducibility of the assay.

Interaction between proteins and DNA is essential for many cellular functions such as DNA replication, DNA repair, maintenance of genomic stability and regulation of gene expression. Transcription is controlled by the association of epigenetically modified histone proteins, chromatin modifiers and transcription factors with target DNA sequences¹. Chromatin immunoprecipitation (ChIP)² has become a prominent technique to analyze protein association with specific sites regions in cells³ in the context of development, differentiation, aging and disease. In the ChIP assay, proteins and DNA are reversibly cross-linked, chromatin is sheared and the protein of interest is selectively immunoprecipitated using specific antibodies. The immunoprecipitated DNA is analyzed for the presence of particular sequences by quantitative polymerase chain reaction (qPCR), hybridization to microarrays (ChIP-chip) or direct sequencing³ (ChIP-seq). Enrichment of specific sequences in the precipitate indicates that these sequences are associated with the protein of interest in vivo.

The Diagenode LowCell# ChIP kit enables the immunoprecipitation in a day's work of up to 8 (or 16) parallel ChIP reactions using one (or two) row(s) respectively of our new designed Magnetic Rack, which allows rapid handling at constant temperature. LowCell# ChIP requires low amounts of reagents per assay including the use of chromatin from low cell numbers as we have shown that 10,000 cells per IP as well as 1,000 cells per IP give similar data in a reproducible manner. The kit also allows immunoprecipitation of histones as well as transcription factors. In addition, it is important to point out that the kit protocol is flexible as it enables down-titration in number of cells needed per IP but on the other hand it is also possible to use up to 100,000 cells per IP. This flexibility is of great help for researchers taking into account that the IP'd DNA amount that is finally obtained matters when it comes to analysis. Less DNA is needed to perform small-scale targeted PCR analysis than larger genome-wide level study.

Principle of LowCell# ChIP

Conventional ChIP assays require large numbers of cells (in the multi-million range), which excludes small and precious cell samples from such analyses. Recent development in the ChIP field has led to the emergence of protocols aiming at reducing cell numbers⁴⁻⁸. The novel LowCell# ChIP kit is unique and includes all major steps of a classical ChIP assay but has been tremendously simplified to minimize sample handling, increase rapidly and reduce the number of steps and kit components. Antibodies to the proteins of interest are first bound to magnetic beads. While binding takes place, cells are collected, DNA-protein interactions are cross-linked by formaldehyde fixation, cells are lysed in a detergent-containing buffer and chromatin is sheared by controlled sonication using the Bioruptor™ (Diagenode). The sheared chromatin is divided into the number of aliquots required by the experiment and incubated with the antibody-coated beads. Immunoselection occurs for 1 hour. The beads, with bound antibody-protein-DNA complexes, are washed, and ChIP'd DNA fragments are purified. DNA sequences are determined by qPCR. A magnetic rack from Diagenode has been specially designed for simple sample handling with the LowCell# ChIP kit (**Fig. 1**). It can hold up to 16 x 0.2-ml tubes simultaneously in a chilled environment even on the bench top, and enables efficient and fast magnetic separation.

Multiple parallel ChIP analysis of histone proteins

The LowCell# ChIP kit allows up to 14 parallel histone immunoprecipitations plus two controls from chromatin prepared from a total of as few as 16,000 cells. For ease of preparation however, 20,000 cells are commonly used as starting material. Reproducibility of ChIP results between large-scale ChIP and low cell number ChIP assays has been documented^{6,7} and extensively validated "in house" with the use of our Bioruptor™, antibodies and LowCell# ChIP kit.

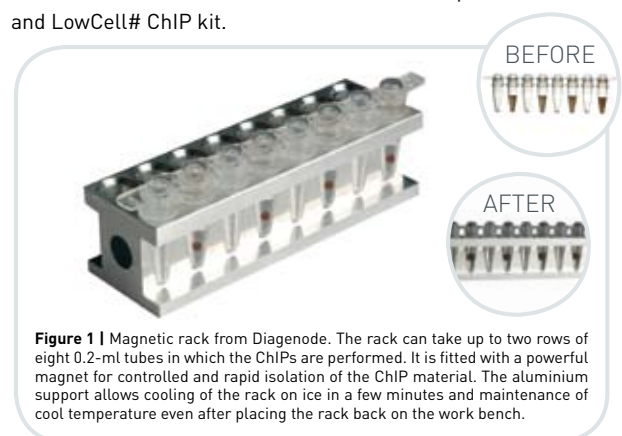


Figure 1 | Magnetic rack from Diagenode. The rack can take up to two rows of eight 0.2-ml tubes in which the ChIPs are performed. It is fitted with a powerful magnet for controlled and rapid isolation of the ChIP material. The aluminium support allows cooling of the rack on ice in a few minutes and maintenance of cool temperature even after placing the rack back on the work bench.

Figure 2 illustrates LowCell# ChIP results using five antibodies directed against epigenetic histone modifications, on the same chromatin sample. Chromatin was prepared from undifferentiated human carcinoma NCCIT cells. ChIP was performed using antibodies directed against trimethylated lysine 4 of histone H3 (H3K4me3), acetylated lysine 9 of histone H3 (H3K9ac), acetylated lysines 9 and 14 of histone H3 (H3K9/14ac) and trimethylated lysine 9 of histone H3 (H3K9me3). Optimized qPCR primers were used to amplify a region of the human *C-FOS* promoter from immunoprecipitated DNA. The data indicate that the *C-FOS* promoter is enriched in H3K4me3, H3K9ac and H3K14ac, in agreement with its expression in NCCIT cells (**Fig. 2**). In contrast, it is not occupied by H3K9me3, an epigenetic mark of inactive loci localized in silent heterochromatin. However, H3K9me3 clearly occupies another genomic region, *SAT2*, in NCCIT cells, which is consistent with this repetitive DNA-containing satellite region being transcriptionally inactive (**Fig. 2**). These results indicate immunoprecipitations in this assay are specific. The low standard deviations between ChIP replicates also illustrate the reproducibility of the assay.

Transcription factor ChIP

Application of the kit to the analysis of DNA-bound transcriptional regulators, such as transcription factors, is also possible. Of note, application of the kit to cell numbers as low as 8,000 cells of starting material (1,000 cells per ChIP) is recommended for relatively abundant transcription factors. For proteins transiently bound to DNA and/or of low abundance, it is preferable to use larger cell numbers, e.g., 100,000 cells as starting material, for ChIPs with chromatin from an equivalent of 10,000 cells. The LowCell# ChIP kit is compatible with these numbers.

Figure 3 shows ChIP results from the immunoprecipitation of the TATA-box binding protein TBP, a transcriptional activator, from undifferentiated NCCIT cells. Association of TBP with three genomic regions, the *C-FOS* promoter, myoglobin exon 2 (*MYO-2*) and the satellite region *SAT2*, is shown. Chromatin from 10,000 cells (**Fig. 3A**) or 1,000 cells (**Fig. 3B**) were used in each ChIP, with 4 µg of anti-TBP antibody. The results indicate that TBP was only bound to the *C-FOS* promoter, in agreement with *C-FOS* being the only transcriptionally active genomic region examined in this experiment. Association of other transcription factors and of RNA polymerase II with specific genomic sites in ChIP assays from low cell numbers has also been reported^{6,7}.

Conclusions

The LowCell# ChIP kit is applicable to both histone and transcription factor analysis. The data are highly reproducible, thus the kit can be used for epigenetic studies of precious cell samples. Downstream applications the kit include analysis of embryos, which so far has only been enabled by carrier ChIP⁴,

small purified stem cell samples and small tissue biopsies. The kit may also be applied to genome-wide studies after a DNA amplification step. Additional information on the LowCell# ChIP kit is available at <http://www.diagenode.com>.

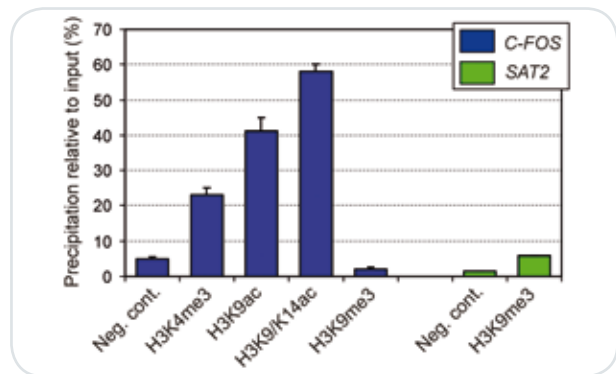


Figure 2 | Histone modifications associated with the *C-FOS* promoter in undifferentiated cells, determined using the LowCell# ChIP Kit. Diagenode antibodies against H3K4me3 (cat. #: pAb-003-024), H3K9ac (cat. #: pAb-004-044), H3K9/14ac (cat. #: pAb-005-044) and H3K9me3 (cat. #: pAb-056-050) were used, each in an aliquot from a chromatin preparation from 1,000 human teratocarcinoma NCCIT cells. Optimized qPCR primers were used to amplify a region of the *C-FOS* promoter (cat. #: pp-1004-050, -500) or the *SAT2* region (cat. #: pp-1040-050, -500), as indicated, from the ChIP'd DNA. One microgram of antibody was used per immunoprecipitation. A negative control antibody was included in the assay (1 µg/ChIP). Data from 3 independent experiments (mean±SD).

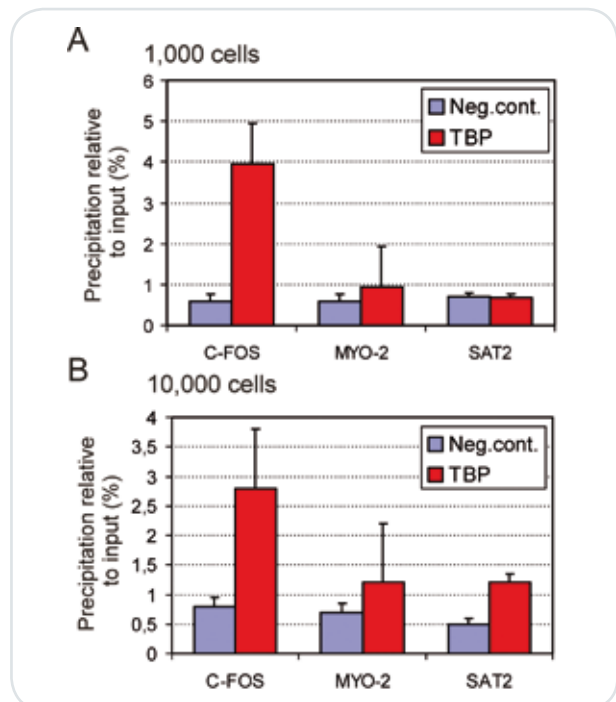


Figure 3 | Assessment of the association of the TATA-box binding protein TBP on various genomic sites in undifferentiated NCCIT cells. TBP was immunoprecipitated using a specific antibody (Diagenode cat. #: MAb-002-100) and qPCR primers to a region of the *C-FOS* promoter (cat. #: pp-1004-050, -500), myoglobin exon 2 (*MYO-2*) (cat. #: pp-1006-050, -500) and *SAT2* (cat. #: pp-1040-050, -500) were used for detection of the ChIP'd DNA. In each ChIP reaction, chromatin from (A) 10,000 cells or (B) 1,000 cells was used with 4 µg anti-TBP antibody. A negative control antibody (4 µg) was included in the assay. Each ChIP was performed in triplicate (mean±SD).

References

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