# SimpleChIP® in Literature

***A synopsis of a recent publication by***
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## Background

Multiple myeloma (MM) is a lethal malignancy in which plasma cells accumulate in the bone marrow. Insulin-like growth factor-1 (IGF-1) and IGF-1 receptor (IGF-1R) are implicated in MM, and expression levels of both are used as negative prognostic factors in diagnosis. IGF-1 induces proliferation via activation of the MAPK/Erk pathway, and increases apoptotic resistance via activation of the PI3K/Akt pathway. The use of IGF-1R blocking agents for MM therapy is currently in clinical trials. Bim, a member of the Bcl2 family, promotes apoptosis via direct or indirect activation of Bax-like family members. Histone deacetylase inhibitors (HDACi) have been shown to induce Bim expression in MM cells. This study demonstrates that IGF-1 treatment of MM cell line Karpas707 down-regulates Bim expression via changes in posttranslational histone modifications.

## Methods and Materials

The human myeloma cell line Karpas707 was used to investigate the epigenetic and posttranslational histone modification state of the *Bim* promoter, using HDACi and DNA demethylating agents.

The chromatin was prepared (Figure 1) and the chromatin immunoprecipitation assay was performed using the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 from Cell Signaling Technology (CST) according to the CST recommended protocol. The chromatin was immunoprecipitated with Normal Rabbit IgG #2729 (negative control), Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb #9649, Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751, Di-Methyl-Histone H3 (Lys9) Antibody #9753, and Histone H3 Antibody (ChIP Formulated) #2650 (positive control).

## Results and Discussion

Gene expression profiling was performed to investigate the effects of IGF-1 treatment in the murine MM cell line 5T33MMvv. Among the most consequential of the down-regulated genes was the proapoptotic gene *Bim*. The silencing of *Bim* in Karpas707 cells by RNA interference conferred partial protection against the HDACi LBH589-induced cell death (data not shown), indicating Bim involvement in drug sensitivity and cell survival in MM cells.

To investigate the effects of HDACi and DNA demethylating agent on the Bim promoter, Karpas707 cells were left untreated (control) or treated with either the DNA demethylation agent decitabine (DAC), the HDACi LBH589, or a combination of both. ChIP analysis of the Bim promoter demonstrated that DAC treatment resulted in reduced acetylation of histone H3 lysine 9 (H3K9Ac) and trimethylation of histone H3 lysine 4 (H3K4Me3). LBH589 treatment resulted in increased H3K9Ac levels, and reduced H3K4Me3 and dimethylation of histone H3 lysine 9 (H3K9Me2). The combination of DAC and LBH589 treatment yielded a marked increase in H3K9Ac and decrease in H3K9Me2 (Figure 2). The most transcriptionally permissive configuration of post-translational modifications at the Bim promoter is high H3K4Me3 and H3K9Ac (permissive) levels, and low H3K9Me2 (repressive) levels. The combination of both DAC and LBH589 treatment resulted in this transcriptionally permissive chromatin state, yielding *Bim* reactivation.

> *"For our ChIP experiments we use the SimpleChIP Kit. Nuclei are efficiently isolated and lysed. The SimpleChIP Kit always works and gives us very reproducible results with a low background."*
>
> *– Claudia Erpelinck, research technician Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands*

ChIP analysis of the *Bim* promoter, in Karpas707 cells after 4 days of IGF-1, demonstrated reduced H3K9Ac levels following IGF-1 treatment, and a small increase in H3K9Me2 levels (Figure 3). This transcriptionally repressive chromatin state indicates that IGF-1 affects Bim expression though histone tail modifications in the promoter region.

These results indicate that in MM, Bim expression is regulated by epigenetics and posttranslational histone modifications. Additionally, IGF-1 was demonstrated to play a role in the epigenetic mechanisms modulating *Bim* gene repression. The findings presented here suggest that the combination of IGF-1R blocking therapies with therapies targeting proapoptotic genes, such as Bim, hold great potential in the advancement of MM treatment.

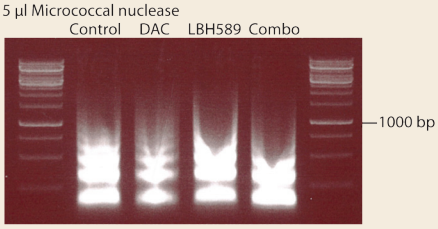


**Figure 1.** Chromatin was prepared according to the protocol provided with the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003, via enzymatic digestion.

ref: De Bruyne E, Bos TJ, Schuit F, Van Valckenborgh E, Menu E, Thorrez L, Atadja P, Jernberg-Wiklund H, and Vanderkerken K. (2010) IGF-1 suppresses Bim expression in multiple myeloma via epigenetic and posttranslational mechanisms. *Blood*. 115, 2430-2440

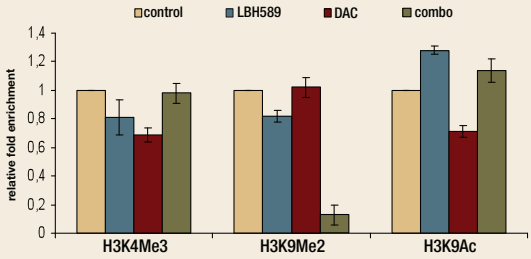


**Figure 2.** ChIP analysis of the Bim promoter in the Karpas707 cells after treatment with LBH589 (HDAC inhibitor) and/or DAC (DNA demethylation agent). Chromatin DNA was immunoprecipitated with either antibodies for tri-methyl-histone H3 lysine 4 (H3K4Me3), di-methyl-histone H3 lysine 9 (H3K9Me2), acetyl-histone H3 lysine 9 (H3K9Ac). Immunoprecipation with normal IgG and histone H3 were used as negative and positive controls, respectively (data not shown). A DNA fragment corresponding to the Bim promoter region was amplified by quantitative real-time PCR.

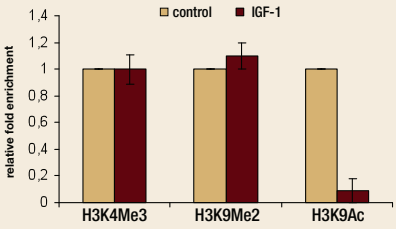


**Figure 3.** ChIP analysis of the Bim promoter in Karpas707 cells after treatment with IGF-1 for 4 days. Chromatin DNA was immunoprecipitated with either antibodies for normal IgG (negative control), trimethyl-histone H3 lysine 4 (H3K4Me3), dimethyl-histone H3 lysine 9 (H3K9Me2), acetyl-histone H3 lysine 9 (H3K9Ac). Immunoprecipation with normal IgG and histone H3 were used as negative and positive controls, respectively (data not shown). A DNA fragment corresponding to the Bim promoter region was amplified by quantitative real-time-PCR.

## Customer Observed Advantages of the SimpleChIP® Kit

- **Increased immunoprecipitation efficiencies:** Most standard ChIP methods utilize sonication, which can either lead to incomplete chromatin fragmentation or "over-sonication" of the sample, causing a loss of antibody epitopes. Using the SimpleChIP® Kit, the chromatin is harvested and fragmented using enzymatic digestion (Figure 1). This micrococcal nuclease digestion is a very important advantage of this kit. We can confirm that the enzymatic digestion leads to a dramatic increase in immunoprecipitation efficiencies.
- **Optimized beads and buffers:** The provided beads and buffers save time because they are optimized to work together, eliminating reagent complications and reducing background.
- **Less variation between experiments:** Chromatin immunoprecipitation is performed using ChIP Grade Protein G Magnetic Beads #9006. These beads do not require any additional blocking, which is convenient and greatly diminishes variation between experiments.
- **Less time consuming:** The elution of the chromatin from the antibody and protein G beads, and reversing the cross-links requires only a 2 hour incubation, saving time compared to other methods.
- **More convenient:** The DNA is isolated with convenient spin columns and ready for any downstream application.
- **The kit includes the positive control Histone H3 Antibody (ChIP formulated) #2650 and negative control Normal Rabbit IgG #2729.**
- **The SimpleChIP® Kit includes control primers** specific to the human or mouse RPL30 gene to facilitate most optimal controls.

*"We are very happy with the SimpleChIP Kit from Cell Signaling Technology as this kit provides us with a very convenient and reliable method for our chromatin studies. As the kit comes with an optimized protocol including all controls, it saves us much time on optimization. With this standardized protocol the results are really reproducible."*

*– Tomas J. Bos, Ph.D., Department of Hematology and Immunology, Vrije Universiteit Brussel, Brussels, Belgium*