

NEW

ChIP Normalization Reagents Reduce Effects of Technical Variation and Reveal Subtle Biological Changes

ChIP is a multi-step process in which variations caused by sample loss during immunoprecipitation and library preparation, uneven sequencing read depth or hand-to-hand differences can lead to results that are difficult to interpret. To overcome this challenge, Active Motif has developed a spike-in strategy that utilizes *Drosophila* chromatin and a *Drosophila*-specific antibody for normalization of technical variation and sample processing bias. Additionally, the normalization strategy can be used for monitoring conditional effects, such as those induced by compounds or mutants, on your experiments.

How does it work?

Active Motif's **Spike-In Normalization Strategy** works with both ChIP-qPCR and ChIP-Seq analysis to eliminate bias and reveal latent biological changes in your samples (Figure 1). ChIP normalization can easily be implemented simply by integrating our **Spike-in reagents** into your standard ChIP protocol.

A standard ChIP reaction is set up using experimental chromatin (e.g. human) and an antibody of interest. In addition, *Drosophila melanogaster* **Spike-in Chromatin** is added, or

"spiked-in", to each reaction as a minor fraction of total chromatin. An antibody that recognizes the *Drosophila*-specific histone variant, H2Av, is also added to the reaction. The **Spike-in Antibody** provides a mechanism to reliably pull down a small fraction of *Drosophila* chromatin that is consistent across all

samples [see [Workflow below](#)].

Since variation introduced during the ChIP procedure will also occur with the Spike-in Chromatin, a normalization factor can be created based on the *Drosophila* signal and applied to the sample genome.

ChIP-Seq Normalization Workflow

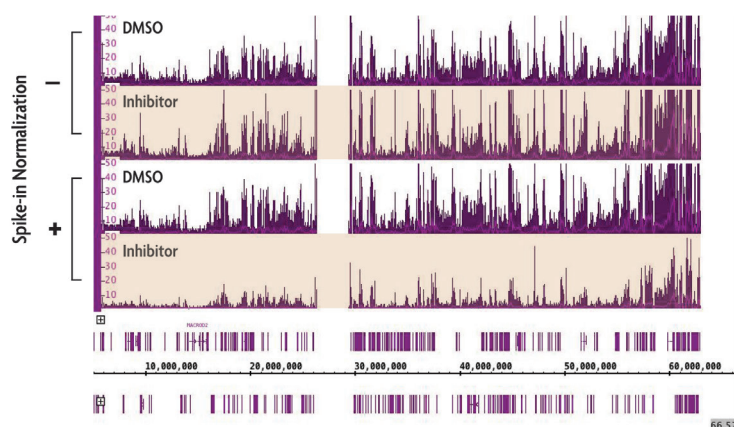
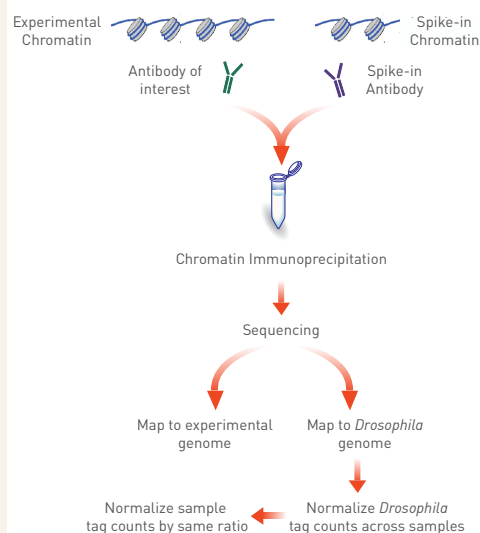


Figure 1: ChIP-Seq Spike-in Normalization Strategy reveals changes in H3K27me3 levels following treatment with EZH2 inhibitor compound. Cells treated with a small molecule inhibitor of EZH2 methyltransferase have dramatic reductions in global H3K27me3 levels. However, H3K27me3 ChIP-Seq using standard ChIP-Seq protocols (-) does not detect these differences. Incorporation of Active Motif's ChIP-Seq Spike-in Strategy (+) reveals the expected decrease in H3K27me3 ChIP-Seq signal.

Product	Format	Catalog No.
Spike-in Chromatin	10 µg	53083
Spike-in Antibody	10 µg	61686
<i>Drosophila</i> Positive Control Primer Set Pbgs	96 rxns	71037
<i>Drosophila</i> Negative Control Primer Set 1	96 rxns	71028