

NEW: Perform ChIP on FFPE Samples with the ChIP-IT® FFPE Kit

Active Motif has a long history of innovation in chromatin immunoprecipitation assays. We develop technologies and utilize quality reagents to make the fastest, easiest, most sensitive ChIP kits available. Because we are able to achieve such a high level of sensitivity, Active Motif has been able to develop a new ChIP-IT® FFPE Chromatin Preparation Kit and a new ChIP-IT® FFPE Kit. These first-of-their-kind products enable extraction of chromatin from FFPE slides or tissue blocks for use in ChIP analysis.

First of its kind assay

Formalin-fixed paraffin-embedded (FFPE) tissue serves as the gold standard for the preservation of pathology samples. As a result, large collections of FFPE samples are commercially available. These samples have the potential to provide valuable information about the epigenetic variances between normal and diseased tissue states.

Traditionally, FFPE samples have not been useful in chromatin immunoprecipitation because of the difficulty in obtaining high-quality chromatin from slides or tissue blocks. The formalin fixation process often causes degradation and loss of antigenicity. With Active Motif's new ChIP-IT® FFPE Chromatin Preparation Kit, high-quality chromatin can be extracted from FFPE samples and, when used in combination with the ChIP-IT FFPE Kit, the assay is sensitive enough to obtain ChIP-enriched DNA for analysis by qPCR or Next-Generation sequencing.

What's in the box?

The ChIP-IT FFPE Chromatin Preparation Kit provides specially formulated reagents and protocol guidelines to extract chromatin from preserved samples, including human FFPE tissues. While the quality of the extracted chromatin is dependent upon sample fixation and storage conditions, we have successfully extracted ChIP-grade chromatin from

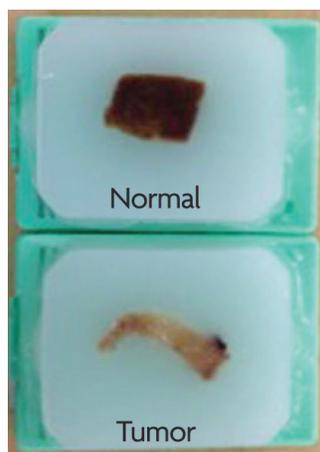


Figure 1: FFPE tissue blocks of normal and tumor samples from human colon stored for over 10 years. Images of the normal and tumor human colon FFPE blocks used with the ChIP-IT FFPE Chromatin Preparation Kit. These samples had been stored in an uncontrolled environment for more than 10 years. Five 20 µm sections from each sample were pooled and processed to obtain enough chromatin for use in the ChIP-IT FFPE Kit.

normal and tumor human colon FFPE blocks that were stored for more than 10 years under less than ideal conditions (Figure 1).

Important considerations

Optimization of extraction conditions will depend on the size and tissue type. Our detailed protocol offers guidelines and troubleshooting tips for processing samples to obtain sufficient chromatin for use in downstream ChIP analysis. The kit also includes positive control DNA and primers to help validate chromatin quality by qPCR analysis prior to proceeding with the ChIP reactions. Table 1 lists examples of the sample material we have successfully validated with the ChIP-IT FFPE Chromatin Preparation Kit.

FFPE sample	Sample used per chromatin prep
Human Colon	Tissue block – five 20 µm sections
Human Kidney	Tissue block – twenty-five 20 µm sections
Human Lung	Tissue block – two 20 µm sections
Rat whole brain	5 slides – two 5 µm sections per slide
Rat hippocampus	25 slides – two 5 µm sections per slide

Table 1: Examples of FFPE samples successfully used with the ChIP-IT FFPE Kit.

Enrich for histone or transcription factor targets with the ChIP-IT FFPE Kit

To perform ChIP analysis on the chromatin extracted from FFPE samples it is necessary to use the new ChIP-IT FFPE Kit. This is the only ChIP Kit available that has the sensitivity required to work with extremely limited starting material while producing minimal background signal, thereby enabling specific detection of the target protein of interest.

Alternative ChIP kits are unable to provide the level of sensitivity required for specific enrichment. The ChIP-IT FFPE Kit includes a positive control antibody to validate successful ChIP reactions. For downstream applications such as Next-Generation sequencing, the ChIP-IT qPCR Analysis Kit is recommended to evaluate the quality of the ChIP-enriched DNA.

ChIP-IT FFPE advantages

- **Obtain quality chromatin** – use slides or tissue blocks as input
- **Sample versatility** – works with human, mouse or rat samples
- **Positive controls** – included in both the chromatin preparation and ChIP kits to validate results at each step of the process
- **Validated** – ChIP-enriched DNA was tested using both qPCR & ChIP-Seq to confirm the enrichment of histone and transcription factor targets

For more complete details, or to download a copy of our product manuals, please visit www.activemotif.com/ffpechip.

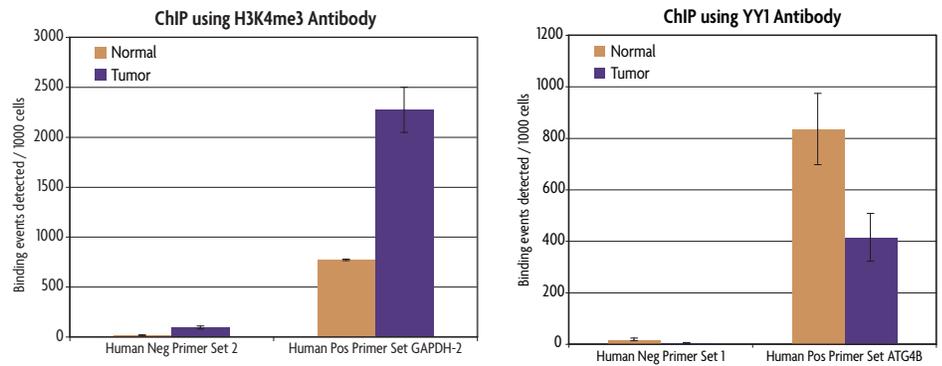


Figure 2: qPCR analysis of normal and tumor human colon samples assayed using ChIP-IT FFPE.

The normal and tumor human colon samples shown in Figure 1 were sliced into 20 µm sections; five sections per sample were pooled and used to prepare one chromatin preparation each with the ChIP-IT FFPE Chromatin Preparation Kit. The chromatin quality was validated using the included positive control DNA and primer sets. 300 ng of normal and tumor chromatin, respectively, were used per ChIP reaction using the ChIP-IT FFPE Kit. Antibodies for histone H3K4me3 or transcription factor YY1 were used for enrichment according to the recommendations in the manual. The quality of the ChIP-enriched DNA was then validated using the ChIP-IT qPCR Analysis Kit, which enables normalization of the data to account for differences in chromatin amounts, primer efficiency and ChIP elution volumes. The qPCR results for each ChIP antibody are shown above. The H3K4me3 results match observed data in which GAPDH is upregulated in certain cancers.

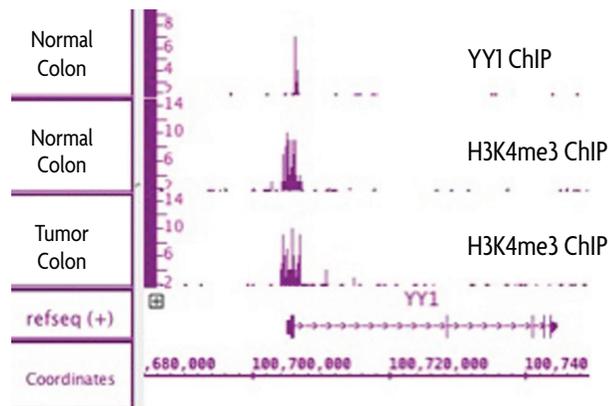


Figure 3: ChIP-IT FFPE ChIP-Seq results using normal and tumor human colon samples.

H3K4me3 ChIP-Seq results from tumor and normal FFPE colon samples and YY1 ChIP-Seq from normal colon are displayed above. Only the portion of the genome-wide data surrounding the YY1 gene is shown. The top panel shows YY1 binding at the promoter of the YY1 gene, thus illustrating the known autoregulation of YY1. The middle and bottom sequence represent H3K4me3 binding at the YY1 gene in normal and tumor, respectively, indicating YY1 is expressed in both the normal and tumor samples.

Product	Format	Catalog No.
ChIP-IT® FFPE Chromatin Preparation Kit	5 rxns	53030
ChIP-IT® FFPE	16 rxns	53045
ChIP-IT® qPCR Analysis Kit	10 rxns	53029