ChIP validated antibodies from CST

We benchmark ourselves against other leading antibody suppliers, so that we may continue to offer the most highly specific and sensitive antibodies available in the marketplace. This iBook provides examples of how our antibodies perform in comparison with antibodies from other providers, and demonstrates how our histone modification antibodies are specially tested to ensure specificity.

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BRD4 (E2A7X) Rabbit mAb #13440

Results:

BRD4 (E2A7X) Rabbit mAb provides better enrichment than the polyclonal antibody from the other company.

Method:

Sample:

4 x 10^6 MV-4-11 cells

ChIP Reagents:

SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

Antibodies:

BRD4 (E2A7X) Rabbit mAb #13440 at 1:50

Other Company’s BRD4 Rabbit pAb at 1:50*

Normal Rabbit IgG #2729 at 1:250

Primers:

SimpleChIP® Human Bcl-2 Promoter Primers #12924

Human c-Myc intron 1 primers

SimpleChIP® Human α Satellite Repeat Primers #4486

Background:

• BRD4 is a member of the bromodomain containing BET family of proteins. It acts as a chromatin-binding protein with a preference for Lys14 on histone H3 as well as Lys5 and Lys12 on histone H4 (1, 2).

• Bromodomain containing proteins like BRD4 regulate the expression of many proteins including those involved in cell growth (e.g., c-Myc) and apoptosis (e.g., Bcl-2) (3, 4).

• α Satellite DNA is found near centromeres and is composed of non-coding, tandem sequence repeats. BRD4 is not significantly bound to this region of the genome.

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**Results:**

As expected, SPT16 (D7I2K) Rabbit mAb enriches for regions of DNA within the open reading frame of c-Fos and Egr1. Enrichment by the other company’s polyclonal antibody is difficult to distinguish from the background.

**Background:**

- Suppressor of Ty-16 (SPT16) is a subunit of the facilitates chromatin transcription (FACT) complex (5, 6). This complex is essential for transcriptional elongation (5).

- The FACT complex facilitates RNA polymerase-dependent transcription of chromatin templates by destabilizing the nucleosomes within the open reading frames of active genes (7–9) like c-Fos and Egr1.

**Method:**

**Sample:**

4 x 10⁶ HCT 116 cells starved for 48 hr then serum stimulated with 20% FBS for 15 min

**ChIP Reagents:**

SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

**Antibodies:**

- SPT16 (D7I2K) Rabbit mAb #12191 at 1:50
- Other Company's SPT16 Rabbit pAb at 1:50*  
- Normal Rabbit IgG #2729 at 1:250

**Primers:**

- SimpleChIP® Human c-Fos Promoter Primers #4663
- SimpleChIP® Human c-Fos Exon 3 Primers #12010
- SimpleChIP® Human EGR1 Promoter Primers #5549
- SimpleChIP® Human EGR1 Intron 3 Primers #11953

*Other Company’s Abs are used in accordance to the manufacturer’s recommended dilution.
**Background:**

- Histone H2A is mono-ubiquitinated at Lys119 by the Polycomb Repressor Complex 1 (PRC1). This modification is critical for transcriptional silencing of the developmental HOX genes (10–13).

- α Satellite DNA is localized near the centromeres and is composed of non-coding, tandem sequence repeats. Histone H2A is not significantly modified in this region of the genome.

**Results:**

The ChIP validated antibody from the other company did not provide target enrichment above background level.

**Method:**

**Sample:**

4 x 10^6 NTERA-2 cells

**ChIP Reagents:**

SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

**Antibodies:**

- Ubiquityl-Histone H2A (Lys119) (D27C4) XP® Rabbit mAb #8240 at 1:50
- Other Company’s Ubiquityl-Histone H2A (Lys119) Mouse mAb (dilution factor as indicated)*
- Normal Rabbit IgG #2729 at 1:250

**Primers:**

- SimpleChIP® Human HoxA1 Intron 1 Primers #7707
- SimpleChIP® Human HoxA2 Promoter Primers #5517
- SimpleChIP® Human α Satellite Repeat Primers #4486

*Other Company’s Abs are used in accordance to the manufacturer’s recommended dilution.*
**Results:**

The CST antibody for c-Myc enriched the ATF4 and NPM1 loci as expected. The ChIP validated antibody from the other company did not provide target enrichment above background level.

**Background:**

- Members of the Myc/Max/Mad network function as bHLH-ZIP transcriptional regulators with roles in various aspects of cell behavior including proliferation, differentiation and apoptosis (14).

- A global survey of the genome using chromatin immunoprecipitation and promoter microarrays demonstrates that ATF4 is a direct target of c-Myc (15).

- Scanning chromatin immunoprecipitation (ChIP-on-chip) experiments were used to show that NPM1 is a direct target of c-Myc (16).

- α Satellite DNA is localized near the centromeres and is composed of non-coding, tandem sequence repeats. c-Myc is not known to bind significantly to these regions of the genome.

**Method:**

**Sample:**

4 x 10⁶ Daudi cells

**ChIP Reagents:**

SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

**Antibodies:**

c-Myc (D3N8F) Rabbit mAb #13987 at 1:50

c-Myc (9E10) Mouse mAb at 1:125

Normal Rabbit IgG #2729 at 1:250

**Primers:**

Human ATF4 Promoter Primers

SimpleChIP® Human NPM1 Intron 1 Primers #4779

SimpleChIP® Human α Satellite Repeat Primers #4486
CREB (D76D11) Rabbit mAb #4820

Results:
CREB (D76D11) Rabbit mAb provides better enrichment, at all loci tested, than the antibody from both Company A and Company B.

Background:
- CREB is a bZIP transcription factor that activates target genes through cAMP response elements. CREB is believed to play a key role in promoting neuronal survival, precursor proliferation, neurite outgrowth, and neuronal differentiation in certain neuronal populations (17–19).

- ALS2 was identified as a gene target of CREB during a genome-wide survey of CREB binding sites (21).

- CREB activation is required for the induction of NR4A3 after neuron stress. This induction is thought to contribute to the neuroprotective effects of CREB (20).

- α Satellite DNA is localized near the centromeres and is composed of non-coding, tandem sequence repeats. CREB is not known to bind significantly to these regions of the genome.

Method:
Sample:
4 x 10⁶ 293 cells treated with Forskolin #3828 (30 μM, 1 hr)

ChIP Reagents:
SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

Antibodies:
CreB (D76D11) Rabbit mAb #4820 at 1:50
CREB (NL904) Rabbit mAb at 1:50
Other Company’s CREB Rabbit pAb at 1:100*
Normal Rabbit IgG #2729 at 1:250

Primers:
Human ALS2 exon 1 primers
SimpleChIP® Human NR4A3 Promoter Primers #4829
SimpleChIP® Human α Satellite Repeat Primers #4486

*Other Company's Abs are used in accordance to the manufacturer's recommended dilution.
Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb #12041

Results:
Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb provides better enrichment after dexamethasone treatment than the antibody from the other company.

Background:
- Glucocorticoid hormones control cellular proliferation, inflammation, and metabolism through their association with the glucocorticoid receptor, GR/NR3C1 (a member of the nuclear hormone receptor superfamily of transcription factors) (22).
- When activated with an agonist such as dexamethasone, GR will translocate into the nucleus and activate transcription at sites like those encoding Metallothionein-2 (MT2A) or Thiamine transporter 1 (SLC19A2).
- α Satellite DNA localized near the centromeres and is composed of non-coding, tandem sequence repeats. The GR is not expected to interact significantly with this region of the genome.

Method:
Sample:
A549 cells were cultured with 5% charcoal-stripped FBS for 3 days and then either left untreated or treated with dexamethasone (100 nM; 1 hr)

ChIP Reagents:
SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

Antibodies:
Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb #12041 at 1:50
Other Company’s Glucocorticoid Receptor Rabbit pAb at 1:50*
Normal Rabbit IgG #2729 at 1:250

Primers:
Human MT2A promoter primers
SimpleChIP® Human SLC19A2 Promoter Primers #7681
SimpleChIP® Human α Satellite Repeat Primers #4486

*Other Company’s Abs are used in accordance to the manufacturer’s recommended dilution.
Results:
NF-κB p65 (D14E12) XP® Rabbit mAb provides better enrichment, at all loci tested, than the antibody from the other company.

Background:
- Transcription factors of the nuclear factor κB (NF-κB)/Rel family play a pivotal role in inflammatory and immune responses (23, 24).

- NF-κB is known to activate transcription of cytokines, like IL-8, in response to stress and to mediate its own activity by activating transcription of the inhibitory IκB-α protein (25).

- α Satellite DNA is localized near the centromeres and is composed of non-coding, tandem sequence repeats. NF-κB p65 is not known to bind significantly to these regions of the genome.

Method:
Sample:
4 x 10⁶ HeLa cells treated with hTNFα (30 ng/ml; 1 hr)

ChIP Reagents:
SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

Antibodies:
NF-κB p65 (D14E12) XP® Rabbit mAb #8242 at 1:100
Other Company’s NF-κB p65 Mouse mAb at 1:250*
Normal Rabbit IgG #2729 at 1:250

Primers:
SimpleChIP® Human IκB-α Promoter Primers #5552
Human IL-8 promoter primers
SimpleChIP® Human α Satellite Repeat Primers #4486

*Other Company’s Abs are used in accordance to the manufacturer’s recommended dilution.
Peptide Array Testing of Histone Modification Antibodies

**Background:**

Our modification-specific histone antibodies are validated with a peptide array assay similar to the one described by Fuchs, S.M., et al. (26). These arrays assess antibody reactivity against known modifications across all histone proteins in a single experiment. This method has the additional benefit of testing the effects of neighboring modifications on the ability of the antibody to detect a single modification site.

**Method:**

Antibodies are tested at three concentrations, as indicated in the diagram, which allows for a more thorough analysis of antibody reactivity.

Peptides containing a methyl-lysine (mono-, di-, or tri-methyl), acetyl-lysine or corresponding unmodified lysine, either alone or in combination with a known neighboring histone modification (e.g., histone H3K4Me3 and H3T3Phos) are spotted onto nitrocellulose as indicated in the diagram.

- **Primary Antibody:** Concentrations as indicated on the diagram.
- **Secondary Antibody:** According to manufacturer’s recommendations (LI-COR, Inc.)
- **Detection:** LI-COR® Odyssey® Infrared Imager
- **Data analysis:** ArrayVision Software (GE Healthcare)
Tri-Methyl-Histone H3 (Lys36) (D5A7) XP® Rabbit mAb is highly specific for Tri-methyl H3 (Lys36) and shows minimal cross-reactivity with di-methyl histone H3 (Lys36).
Di-Methyl-Histone H3 (Lys36) (C75H12) Rabbit mAb #2901

Di-Methyl-Histone H3 (Lys36) (C75H12) Rabbit mAb is highly specific for Di-methyl H3 (Lys36).
Acetyl-Histone H4 (Lys16) (E2B8W) Rabbit mAb is highly specific for Acetyl-H4 (Lys16) and shows minimal cross-reactivity with other histone modifications.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>H3 (Lys4)</td>
</tr>
<tr>
<td>B</td>
<td>H3 (Lys4) Acetyl</td>
</tr>
<tr>
<td>C</td>
<td>H3 (Lys9/Lys14/Lys18)</td>
</tr>
<tr>
<td>D</td>
<td>H3 (Lys9) Acetyl</td>
</tr>
<tr>
<td>E</td>
<td>H3 (Lys14) Acetyl</td>
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<tr>
<td>F</td>
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<td>G</td>
<td>H3 (Lys23)</td>
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<td>H3 (Arg26) Asymmetric-dimethyl/lys27 Acetyl</td>
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<td>H4 (Lys12) Lys18/Lys14/Lys16</td>
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<td>KK</td>
<td>H2B (Lys5/Lys12/Lys15/Lys20)</td>
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<td>MM</td>
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<tr>
<td>NN</td>
<td>H2B (Lys15) Acetyl</td>
</tr>
<tr>
<td>OO</td>
<td>H2B (Lys20) Acetyl</td>
</tr>
</tbody>
</table>
## SimpleChIP Kits

- SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) #9002
- SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003
- SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads) #9004
- SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

## Beads and Magnetic Rack

- ChIP-grade Protein G Magnetic Beads #9006
- ChIP-grade Protein G Agarose Beads #9007
- 6-Tube Magnetic Separation Rack #7017

## Individual Kit Components

- Micrococcal Nuclease #10011
- SimpleChIP® DNA Purification Kit
- SimpleChIP® Chromatin Preparation Buffers
- SimpleChIP® Lysis Buffers A and B

### SimpleChIP Kit Components Listed by Protocol Step:

<table>
<thead>
<tr>
<th>Chromatin Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease Inhibitor Cocktail (200X)</td>
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<tr>
<td>1 M DTT</td>
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<tr>
<td>10X ChIP Buffer</td>
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<tr>
<td>Glycine Solution (10X)</td>
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<tr>
<td>Buffer A (4X)</td>
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<tr>
<td>Buffer B (4X)</td>
</tr>
<tr>
<td>Micrococcal Nuclease #10011 (2000 gel units/μl)</td>
</tr>
<tr>
<td>0.5 M EDTA</td>
</tr>
<tr>
<td>RNase A</td>
</tr>
</tbody>
</table>

### Immunoprecipitation

- Protease Inhibitor Cocktail (200X)
- ChIP Buffer (10X)
- Histone H3 (D2B12) XP® Rabbit mAb #4620
- Normal Rabbit IgG (1 μg/μl) #2729
- ChIP-Grade Protein G Beads
- 5 M NaCl

### Cross-link Reversal and DNA Isolation

- 5 M NaCl
- ChIP Elution Buffer (2X)
- Proteinase K (20 mg/ml)
- DNA Binding Reagent A
- DNA Wash Reagent B
- DNA Elution Reagent C
- DNA Spin Columns

### Downstream Analysis

- SimpleChIP® Human RPL30 Exon 3 Primers 1
- SimpleChIP® Mouse RPL30 Intron 2 Primers 1

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References