

Certificate of Analysis

EZ ChIP™ Chromatin Immunoprecipitation Kit

Catalog # 17-371

Lot # 46010

Kit Components

Protein G Agarose ChIP Blocked, Catalog#16-201C, Lot # JBC1366928. One vial containing **1.5 mL** packed beads ChIP Blocked with 1.5 mg BSA and approximately 4.5 mg recombinant Protein G. Provided as a 50% gel slurry for a final volume of **3 mL** per vial. Suspended in TE buffer, pH 8.0, containing 0.05% sodium azide. Liquid suspension.

ChIP Dilution Buffer, Catalog # 20-153, Lot # 0611045659. One vial containing **24 mL** of 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl.

Low Salt Immune Complex Wash Buffer, Catalog # 20-154, Lot # 0609040614. One vial containing **24 mL** of 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl.

High Salt Immune Complex Wash Buffer, Catalog # 20-155, Lot # 0609040615. One vial containing **24 mL** of 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl.

LiCl Immune Complex Wash Buffer, Catalog # 20-156, Lot # JBC1360319. One vial containing **24 mL** of 0.25M LiCl, 1% IGEPAL-CA630, 1% deoxycholic acid (sodium salt), 1 mM EDTA, 10 mM Tris, pH 8.1.

TE Buffer, Catalog # 20-157, Lot # 0701050834. Two vials, each containing **24 mL** of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.

0.5M EDTA, Catalog # 20-158, Lot # JBC1360320. One vial containing **250 µL** of 0.5M EDTA, pH 8.0.

5M NaCl, Catalog # 20-159, Lot # 0605030422. One vial containing **500 µL** of 5M NaCl.

SDS Lysis Buffer, Catalog # 20-163, Lot # JBC1360324. One vial containing **10 mL** of 1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.1.

1M Tris-HCl, pH 6.5, Catalog # 20-160, Lot # 0610042038. One vial containing **500 µL** of 1M Tris-HCl, pH 6.5.

10X PBS, Catalog # 20-281, Lot # JBC1360327. One vial containing **24 mL** of 10X PBS.

10X Glycine, Catalog # 20-282, Lot # JBC1350039. One vial containing **11 mL** of 1.25M Glycine.

Protease Inhibitor Cocktail II, Catalog # 20-283, Lot # 0703054489. Two vials, each containing **110 µL** of 200X Protease Inhibitor Cocktail II in DMSO.

RNase A, Catalog # 20-297, Lot # 0703054490. One vial containing **600µg** of RNase A in **60 µL** of sterile water.

Proteinase K, Catalog # 20-298, Lot # 0701050844. One vial containing **600 µg** of Proteinase K in **60 µL** of 50 mM Tris-HCl, pH 8.0, 10 mM CaCl₂.

1M NaHCO₃, Catalog # 20-296, Lot # 0605030939. One vial containing **600 µL** of 1M NaHCO₃.

Control Primers, Catalog # 22-004, Lot # 0605030943. One vial containing **75 µL** of 5µM of each control primer specific for human GAPDH.

FOR: 5'-TACTAGCGGTTTTACGGGCG-3'

REV: 5'-TCGAACAGGAGGAGCAGAGAGCGA-3'

Anti-acetyl-Histone H3 (Rabbit polyclonal IgG), Catalog # 06-599B, Lot # 0704056838. One vial containing **125µg** of protein A purified IgG in **125 µl** of 0.1M Tris-glycine, pH 7.4, 0.15M sodium chloride with 0.05% sodium azide.

Normal Rabbit IgG, Catalog # PP64B, Lot # 0703054683. One vial containing **125 µg** of normal rabbit IgG.

20% SDS, Catalog # 20-280, Lot # 0605030423. One vial containing **242 µL** of 20% SDS.

Spin Filters, Catalog # 20-290, Lot # 46000. One bag containing 22 Spin Filters in Collection Tubes.

Collection Tubes, Catalog # 20-291, Lot # 46000. One bag containing 22 Collection Tubes.

Bind Reagent A, Catalog # 20-292, Lot # 46000. One vial containing **25 mL** of Bind Reagent A.

Wash Reagent B, Catalog # 20-293, Lot # 46000. One vial containing **12.5 mL** of Wash Reagent B.

Elution Reagent C, Catalog # 20-294, Lot # 46000. One vial containing **1.5 mL** of Elution Reagent C.

**FOR RESEARCH USE ONLY
NOT RECOMMENDED OR INTENDED FOR DIAGNOSIS OF DISEASE IN HUMANS
DO NOT USE IN HUMANS**

Kit Description

Quantity: Two boxes containing the necessary reagents to perform 22 chromatin immunoprecipitation (ChIP) assays. This kit also contains reagents to generate chromatin from ten 10-cm plates to allow for multiple immunoprecipitations from the same chromatin preparation.

Storage and Stability: Upon receipt, store components at the temperatures indicated on the labels. Kit components are stable for 1 year from date of shipment when stored as directed.

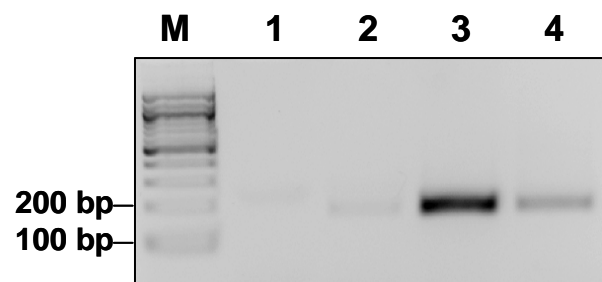
Use: The EZ ChIP™ kit contains reagents optimized for immunoprecipitation of chromatin from mammalian cells, including controls to ensure successful performance of this assay. Detection of the DNA region, gene or promoter of interest in immunoprecipitated chromatin must be empirically determined by the researcher. Quantitative PCR using promoter-specific primers is recommended. The EZ ChIP™ kit has all the necessary buffers and reagents to perform successful chromatin immunoprecipitation assays. However, careful attention must be paid to the details of the instructions. Follow all instructions carefully, especially with regard to incubation times and temperatures.

Please refer to the Instruction Manual for further information and a detailed assay protocol.

Quality Control Testing

PCR Analysis of Chromatin Immunoprecipitation:

Chromatin immunoprecipitation was performed using chromatin from HeLa cells and either anti-acetyl histone H3 (Catalog # 06-599) or Normal Rabbit IgG (Catalog # PP64B) as the immunoprecipitating antibody. Purified DNA was then analyzed by PCR using Control Primers specific for the GAPDH promoter. PCR product was observed in the anti-acetyl H3 ChIP (lane 3) and substantially less was detected in the Normal Rabbit IgG ChIP (lane 2). GAPDH promoter specific DNA was also observed in the 2% Input (lane 4) and not in the "No DNA" PCR control (lane 1).



General References:

1. Das, Partha M, *et al* (2004). Chromatin immunoprecipitation assay. *BioTechniques* **37**: 961-9.
2. Luo, R X, *et al* (1998). Rb interacts with histone deacetylase to repress transcription. *Cell* **92**: 463-73.
3. Braunstein, M, *et al* (1996). Efficient transcriptional silencing in *Saccharomyces cerevisiae* requires a heterochromatin histone acetylation pattern. *Mol Cell Biol* **16**: 4349-56..
4. Buck, Michael J and Lieb, Jason D (2004). ChIP-chip: considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments. *Genomics* **83**: 349-60.
5. Bernstein, Bradley E, *et al* (2004). The use of chromatin immunoprecipitation assays in genome-wide analyses of histone modifications. *Meth Enzymol* **376**: 349-60.
6. Weinmann, Amy S, *et al* (2002). Isolating human transcription factor targets by coupling chromatin immunoprecipitation and CpG island microarray analysis. *Genes Dev* **16**: 235-44.
7. Manabe, I and Owens, G K (2001). CArG elements control smooth muscle subtype-specific expression of smooth muscle myosin in vivo. *J Clin Invest* **107**: 823-34.
8. Cervoni, N and Szyf, M (2001). Demethylase activity is directed by histone acetylation. *J Biol Chem* **276**: 40778-87.