

upstate

now part of Millipore

Certificate of Analysis

28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 800 437-7500 • F: 800 437-7502
email: techserv@upstate.com
www.upstate.com

EZ-Zyme™ Chromatin Prep Kit

Catalog # 17-375

Lot # JBC1369227

Kit Components

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

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

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

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

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

EZ-Zyme™ Lysis Buffer, Catalog # 20-312. One vial containing **2.0 mL** of EZ-Zyme Lysis Buffer.

EZ-Zyme™ Digestion Buffer, Catalog # 20-313. One vial containing **1.65 mL** of EZ-Zyme Digestion Buffer.

EZ-Zyme™ Stop Buffer, Catalog # 20-315. One vial containing **1.65 mL** of EZ-Zyme Stop Buffer.

Protease Inhibitor Cocktail II, Catalog # 20-283. One vial containing **110 µL** of Protease Inhibitor Cocktail II in DMSO.

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

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

EZ-Zyme™ Enzymatic Cocktail, Catalog # 20-314. One vial containing **76U** of Enzymatic Cocktail in 38 µL of a solution containing 50% glycerol.

**FOR IN VITRO 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.

Storage and Stability: Upon receipt, store components at the temperatures indicated on the labels. Kit components are stable for 6 months from date of shipment when stored as directed.

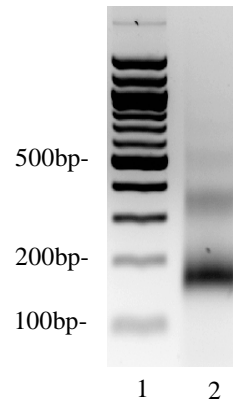
Use: The EZ-Zyme™ kit contains reagents optimized for shearing chromatin from mammalian cells for Chromatin Immunoprecipitation (ChIP) Assay. Detection of the DNA region, gene or promoter of interest in immunoprecipitated chromatin must be empirically determined by the researcher. Quantitative PCR using promoter-specific primers is recommended. The EZ-Zyme™ Chromatin Preparation kit has all the necessary buffers and reagents to perform successful chromatin shearing. 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

Chromatin Digestion

Chromatin from formaldehyde-crosslinked HeLa cells was prepared and digested by following Section A, Section B and Appendix A (steps 12-17) in the instruction manual. 20 μ L of digested chromatin (lane 2) was electrophoresed through a 2% agarose gel and stained with ethidium bromide. Lane 2 shows that the majority of the chromatin has been digested to lengths of mono- and dinucleosomes.



PCR Analysis of Chromatin Immunoprecipitation:

Chromatin immunoprecipitations were performed using digested chromatin from HeLa cells and either anti-Acetylated Histone H3 (Catalog # 06-599) or Normal Rabbit IgG (Catalog #12-370) as the immunoprecipitating antibody. Purified DNA was then analyzed by PCR using Control Primers specific for the β -actin promoter. PCR product was observed in the anti-Acetylated Histone H3 ChIP (lane 4) and not in the Normal Rabbit IgG ChIP (lane 3). β -actin promoter primer specificity was also observed in the input (lane 5) and not in the "No DNA" PCR control (lane 2)

