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Salmon Sperm DNA/Protein A Agarose

Catalog # 16-157
Lot # JBC1349698

Product Description: Recombinant Protein A covalently bound to agarose by alkylamine linkage. Sonicated salmon sperm DNA is included to block non-specific DNA binding sites on protein A agarose when collecting immunocomplexes for chromatin immunoprecipitations (ChIP assays).

Quantity and Formulation: **2.5 mL** packed beads containing **1 mg** sonicated Salmon Sperm DNA, 2.5 mg BSA and approximately 7.5 mg recombinant Protein A. Provided as a 50% gel slurry for a final volume of **5 mL**. Suspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 containing 0.05% sodium azide. Liquid suspension.

Storage and Stability: Stable for 1 year at 4°C from date of shipment.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Chromatin Immunoprecipitation: 80 μL of this lot of gel slurry pre-cleared a chromatin solution and subsequently 60 μL immunoprecipitated DNA cross-linked to acetylated histones using anti-Acetyl Histone H3 (Catalog # 06-599). Testing was performed in conjunction with the Acetyl Histone H4 ChIP Assay Kit (Catalog # 17-229) or EZ-ChIP (Catalog # 17-371). This agarose has also been tested with Acetyl Histone H3 ChIP Assay Kit (Catalog # 17-245).

Application References:

1. Mayo, M. W., *et al* (2003). Ineffectiveness of histone deacetylase inhibitors to induce apoptosis involves the transcriptional activation of NF-kappa B through the Akt pathway. *J Biol Chem* **278**: 18980-9.

2. Tomita, A., *et al* (2003). Fusion Protein of Retinoic Acid Receptor {alpha} with Promyelocytic Leukemia Protein or Promyelocytic Leukemia Zinc Finger Protein Recruits N-CoR-TBLR1 Corepressor Complex to Repress Transcription in Vivo. *J Biol Chem* **278**: 30788-30795.

CHROMATIN IMMUNOPRECIPITATION PROTOCOL

Part A. Optimization of DNA Shearing

Establish optimal conditions required for shearing cross-linked DNA to 200-1000 base pairs in length by following steps 1- 9 below. Vary the power setting and/or the number of 10-second pulses during sonication of the samples. Be sure to keep the sample on ice at all times (the sonication generates heat which will denature the DNA). Check the size of sonicated DNA by gel electrophoresis after reversion of cross-links. Our experience shows DNA is sheared to the appropriate length with 3-4 sets of 10-second pulses using a Cole Parmer, High Intensity Ultrasonic Processor/Sonicator, 50-watt model equipped with a 2mm tip and set to 30% of maximum power. **Once sonication conditions have been optimized, keep cell number consistent for subsequent experiments. The protocol below for the optimization of DNA Shearing is for one Chip assay (~1 x 10^6 cells per condition).**

Note: Steps 3- 7 should be done on ice.

1. Stimulate or treat 1 x 10^6 cells on a 10cm dish as appropriate. (Cells should be treated under conditions for which transcriptional activation of the gene of interest has been demonstrated). Include one extra dish (1 X 10^6 cells) to be used solely for estimation of cell number.
2. Cross link histones to DNA by adding formaldehyde directly to culture medium to a final concentration of 1% and incubate for 10 minutes at 37°C. (For example, add 270 µL 37% formaldehyde into 10 mL of growth medium on plate).
3. Aspirate medium, removing as much medium as possible. Wash cells twice using ice cold PBS containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride (PMSF), 1 µg/mL aprotinin and 1 µg/mL pepstatin A). **Note:** Add protease inhibitors to PBS just prior to use. PMSF has a half-life of approximately 30 minutes in aqueous solutions.
4. Scrape cells into conical tube.
5. Pellet cells for 4 minutes at 2000 rpm at 4°C. Warm **SDS Lysis Buffer (Catalog # 20-163)** to room temperature to dissolve precipitated SDS and add protease inhibitors (inhibitors: 1mM PMSF, 1µg/ml aprotinin and 1 µg/mL pepstatin A).
6. Resuspend cell pellet in 200 µL of **SDS Lysis Buffer (Catalog # 20-163)** and incubate for 10 minutes on ice. **Note:** The 200 µL of SDS Lysis Buffer is per 1 X 10^6 cells; if more cells are used, the resuspended cell pellet should be divided into 200 µL aliquots so that each 200µl aliquot contains ~1 X 10^6 cells.
7. Sonicate lysate to shear DNA to lengths between 200 and 1000 basepairs being sure to keep samples ice cold (***Note:*** Once sonication conditions have been optimized following steps 1 to 9, proceed to Part B, step 1 below).
8. Add 8 µL **5 M NaCl (Catalog # 20-159)** and reverse crosslinks at 65°C for 4 hours.
9. Recover DNA by phenol/chloroform extraction and run sample (example 5 µL, 10 µL and 20 µL samples) in an agarose gel to visualize shearing efficiency.

Part B. Experimental protocol.

If sonication conditions have been optimized (Part A), complete steps 1 through 7 and continue with the protocol below. For a negative/background control, prepare a sample to use as a no-antibody immunoprecipitation control in step 5 below. Additionally, transcriptionally unactivated DNA samples should be prepared as controls for PCR in section II.

1. Centrifuge samples (part A, step 7) for 10 minutes at 13,000 rpm at 4°C, and add 200µl of the sonicated cell supernatant to a new 2ml-microcentrifuge tube. Discard pellet.
2. Dilute the sonicated cell supernatant 10 fold in **ChIP Dilution Buffer (Catalog # 20-153)**, adding protease inhibitors as above. This is done by adding 1800 µL ChIP Dilution Buffer to the 200 µL sonicated cell supernatant for a final volume of 2 mL in each immunoprecipitation condition. **Note:** *If proceeding to PCR a portion of the diluted cell supernatant 1% (~20µl) can be kept to quantitate the amount of DNA present in different samples at the PCR protocol, Part B, section II, step 6. This sample is considered to be your input/starting material material and needs to have the Histone-DNA crosslinks reversed by heating at 65°C for 4 hours (see section II, step 3.).*

3. To reduce nonspecific background, pre-clear the 2 mL diluted cell supernatant with 80 μL of **Salmon Sperm DNA/Protein A Agarose-50% Slurry** for 30 minutes at 4°C with agitation.

4. Pellet agarose by brief centrifugation and collect the supernatant fraction.

5. Add the immunoprecipitating antibody (the amount will vary per antibody) to the 2 mL supernatant fraction and incubate overnight at 4°C with rotation. *For a negative control, perform a no-antibody immunoprecipitation by incubating the supernatant fraction with 60 μL of **Salmon Sperm DNA/Protein A Agarose-50% Slurry** for one hour at 4°C with rotation and proceed to step 7.*

6. Add 60 μL of **Salmon Sperm DNA/Protein A Agarose Slurry** for one hour at 4°C with rotation to collect the antibody/histone complex.

7. Pellet agarose by gentle centrifugation (700 to 1000 rpm at 4°C, ~1min). Carefully remove the supernatant that contains unbound, non-specific DNA. Wash the protein A agarose/antibody/histone complex for 3-5 minutes on a rotating platform with 1 mL of each of the buffers listed in the order as given below:

   a) Low Salt Immune Complex Wash Buffer (Catalog # 20-154), **one wash**
   b) High Salt Immune Complex Wash Buffer (Catalog # 20-155), **one wash**
   c) LiCl Immune Complex Wash Buffer (Catalog # 20-156), **one wash**
   d) 1X TE (Catalog # 20-157), **two washes**

After step 7 above, the sample is now a <u>protein A/antibody/histone/DNA complex</u> ready for either an Immunoprecipitation/Immunoblot assay (Section I) or Polymerase Chain Reaction (PCR) assay (Section II):

Section I. Immunoprecipitation/Immunoblot protocol to detect histone.

1. Following washing of the beads in part B, step 7, immunoprecipitated histones can be analyzed by immunoblot analysis. Add 25 μL of 1X Laemmli buffer per sample and boil for 10 minutes. Load 20 μL per lane and perform immunoblot procedure as described per appropriate antibody.

Section II. PCR protocol to amplify DNA that is bound to the immunoprecipitated histone.

1. Freshly prepare elution buffer (1%SDS, 0.1M $NaHCO_3$).

2. Elute the histone complex from the antibody by adding 250 μL elution buffer to the pelleted protein A agarose/antibody/histone complex from step 7d above. Vortex briefly to mix and incubate at room temperature for 15 minutes with rotation. Spin down agarose, and carefully transfer the supernatant fraction (eluate) to another tube and repeat elution. Combine eluates (total volume = ~500 μL).

3. Add 20 μL 5 M NaCl (Catalog # 20-159) to the combined eluates (500 μL) and reverse histone-DNA crosslinks by heating at 65°C for 4 hours. At this step the sample can be stored and -20°C and the protocol continued the next day.

   Note: Include the input/starting material (*the sample saved from Part B, step 2, which has had the Histone-DNA crosslinks reversed*)) as well as a transcriptionally-unactivated DNA sample as negative and background controls for the PCR reaction. *Previously, a 5 μL sample has been used in a nested PCR reaction. However, the amount of sample used per reaction must be determined empirically (e.g., titrate the sample at this step by using 1, 2, 5, or 10 μL per PCR reaction)*. If PCR results are poor, <u>complete steps 4, 5 and 6 below to purify the DNA sample</u>. NOTE: Handle the samples carefully, some DNA may be lost during the purification steps.

4. Add 10 μL of 0.5 M EDTA (Catalog # 20-158), 20 μL 1M Tris-HCl, pH 6.5 (Catalog # 20-160) and 2 μL of 10 mg/mL Proteinase K to the combined eluates and incubate for one hour at 45°C.

5. Recover DNA by phenol/chloroform extraction and ethanol precipitation. Addition of an inert carrier, such as 20 μg glycogen or yeast tRNA, helps visualize the DNA pellet. Wash pellets with 70% ethanol and air dry.

6. Resuspend pellets in an appropriate buffer for PCR or slot-blot reactions. PCR or slot-blot conditions must be determined empirically.