Certificate of Analysis

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Salmon Sperm DNA/Protein A Agarose

(50% slurry)
Catalog # 16-157
Lot # 17124

Quantity and Formulation: Five vials, each vial containing **500ml** packed beads containing **200mg** sonicated salmon sperm DNA, 500µg BSA and approximately 1.5mg recombinant Protein A. Provided as a 50% gel slurry for a final volume of 1ml. Suspended in 1X TE buffer containing 0.05% sodium azide.

Physical Form: 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: 80µl of this lot of gel slurry pre-cleared a chromatin solution and subsequently 60µl of this lot immunoprecipitated DNA cross-linked to acetylated histones using anti-Acetyl Histone H4, ChIPs Grade (Catalog # 06-866). Testing was performed in conjunction with the Acetyl Histone H4 ChIP Assay Kit (Catalog # 17-229).

Background: Recombinant Protein A covalently bound to agarose by alkylamine linkage. Sonicated salmon sperm DNA blocks non-specific DNA binding sites on protein A agarose when collecting immunocomplexes for chromatin immunoprecipitations (ChIPs assays).

Chromatin Immunoprecipitation Protocol

Perform a mock experiment (steps 1-5) to establish optimal conditions required to shear cross-linked DNA to 200-1000 basepairs in length. Vary the power setting and/or the number of 10 second pulses during sonication of the samples. Chill samples between pulses . Check the size of sonicated DNA by gel electrophoresis after reversion of cross-links (step 14). Our experience shows DNA is sheared to the appropriate length with 3 sets of 10 second pulses using a Vibra cell sonicator equipped with a 2mm tip and set to 30% of maximum **power.**

1. Stimulate or treat 0.5-2 x 10^6 cells on 10cm dish as appropriate.
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. Seal culture vessels if returning the cultures to an incubator containing other cells.
3. Aspirate medium, wash and scrape cells with ice cold PBS containing protease inhibitors (1mM phenylmethylsulfonyl fluoride [PMSF], 1µg/ml aprotinin and 1µg/ml pepstatin A). We recommend using PMSF stock solutions less than 1 month old and adding PMSF to the buffer just prior to use because PMSF has a half-life of about 30 minutes in aqueous solutions.
4. Pellet cells for 4 minutes at 700 x g at 4°C. Resuspend cell pellet in 200µl SDS lysis buffer (1% SDS, 10mM EDTA, 50mM Tris-HCl, pH 8.1) for 10 minutes on ice.
5. Sonicate lysate to reduce DNA length to between 200 and 1000 basepairs. Cool samples on dry ice between pulses but do not freeze the samples. Remove debris by centrifugation for 10 minutes at 13,000 rpm at 4°C in a microcentrifuge.
6. Dilute supernatant fraction 10 fold in ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2mM EDTA, 16.7mM Tris, pH 8.1, 16.7mM NaCl and protease inhibitors). Keep a portion of this chromatin solution (1%) to check the amount of input DNA present in different samples before immunoprecipitation.
7. To reduce nonspecific background, pre-clear the chromatin solution with **80µl of Salmon Sperm DNA/Protein A Agarose** for 30 minutes at 4°C with agitation.
8. Pellet beads by a brief centrifugation and collect supernatant fraction.
9. Add 5µl of anti-acetyl Histone H4, ChIPs Grade (Catalog # 06-866) to 1ml of chromatin solution, the supernatant fraction collected in step 8, incubate overnight at 4ºC with rotation. Save the other 1ml of chromatin solution for a no-antibody control.
10. Collect immunocomplexes with **60µl of Salmon Sperm DNA/Protein A Agarose** for 1 hour at 4ºC with rotation.
11. Pellet beads by centrifugation and wash five times using the sequence of buffers listed below. Use 1ml of buffer per wash, 3-5 minutes each:
    a) 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1, 150mM NaCl.
    b) 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1, 500mM NaCl.
    c) 0.25M LiCl, 1% NP40, 1% sodium deoxycholate, 1mM EDTA, 10mM Tris-HCl, pH 8.1.
    d) TE, pH 8.0.
    e) TE, pH 8.0.
12. Elute immunocomplexes by adding 250µl 1% SDS in 0.1M $NaHCO_3$ to pelleted beads. Vortex briefly to mix and incubate at room temperature for 15 minutes with rotation. Spin down beads, carefully transfer the supernatant fraction (eluate) to another tube and repeat elution. Combine eluates.
13. Add 20µl 5M NaCl to the combined eluates and reverse crosslinks at 65ºC for 4 hours. **NOTE:** 5µl of this material can be used in nested-PCR to determine if the gene/promotor of interest was immunoprecipitated.
14. Add 10µl of 0.5M EDTA, 20µl 1M Tris-HCl, pH 6.5, and 2µl of 10mg/ml Proteinase K to the eluate and incubate for 1 hour at 45°C.
15. Recover DNA by phenol/chloroform extraction and ethanol precipitation using 20µg glycogen as a carrier.
16. Detect specific sequences from immunoprecipitated and input DNA by quantitative PCR or slot-blot. Conditions must be determined empirically