Assays of nucleosome assembly and the inhibition of histone acetyltransferase activity. (3) Preparation of nuclei from HeLa cells

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Method Article

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Abstract

Introduction

For a detailed introduction to assays of nucleosome assembly and the inhibition of histone acetyltransferase activity, please go here: "http://www.natureprotocols.com/2007/07/30/assays_of_nucleosome_assembly.php":http://www.natureprotocols.com/2007/07/30/assays_of_nucleosome_

Reagents

 Phosphate-buffered saline \(PBS; pH 7.4) Nucleus-isolation buffer: 10 mM Tris-HCl \(pH 7.5), 1.5 mM $MgCl_2$, 1.0 mM $CaCl_2$, 0.25 M sucrose \(Wako Pure Chemical Industries, Ltd.; cat. no. 196-00018), 0.1 mM phenylmethylsulfonyl fluoride \(PMSF; Sigma-Aldrich Japan; cat. no. L3771) 20 % \(v/v) Triton X-100 \(Sigma-Aldrich Corp.; cat. no. 93426) Saturated solution of NaCl and urea \[5 M NaCl, 8 M urea \(Sigma-Aldrich Corp.; cat. no. 02493; stirred overnight and stored at room temperature)] Dounce homogenizer \(B pestle; Wheaton Science Products, Millville, NJ, U.S.A.; cat. no. 357542)

Procedure

1. Harvest HeLa S3 cells from a culture of approximately 5 x 10^5 cells/mL in a 2-L spinner flask by centrifugation at 500 x g for 10 min at 4 °C. 2. Wash the cell pellet by gentle resuspension in ice-cold PBS and repeat the centrifugation as above. 3. Wash the cell pellet again with ice-cold nucleus-isolation buffer and resuspend the cell pellet by gentle agitation in 50 mL of ice-cold nucleus-isolation buffer supplemented with 0.5% \(v/v) Triton X-100. Allow the cells to swell for 10 min on ice. 4. Homogenize the swollen cells on ice with 15 strokes of a Dounce homogenizer on ice. Pour the suspension of homogenized cells \(about 60 mL) into two 50-mL conical tubes and centrifuge them at 500 x g for 5 min at 4 °C. 5. Pour off cloudy supernatants slowly and then gently resuspend each nuclear pellet in approximately 5 mL of nucleus-isolation buffer plus Triton X-100. Add nucleus-isolation buffer to bring the suspension to the original volume \(approximately 30 mL per tube) and pellet the nuclei again by centrifugation at 500 x g for 5 min at 4 °C. 6. Repeat step 5, at least twice, until the nuclei are pure white and the supernatant is clear. 7. Resuspend each nuclear pellet gently in approximately 5 mL of nucleus-isolation buffer and combine the suspensions in one conical tube. Adjust the total volume to approximately 20 mL with nucleus-isolation buffer. Remove a 5-µL aliquot and measure the absorbance at 260 nm in 1 mL of a saturated solution of NaCl and urea, using this solution as a blank, to quantify the amount of chromatin DNA \(20 OD_{260nm} = 1 mg/mL DNA). The total yield should be close to 30 mg of chromatin as DNA. Pellet the nuclei by centrifugation at 10,000 x g and carefully remove the supernatant. Freeze the nuclear pellet in liquid nitrogen and store at −80 ° C.