Chromatin Immunoprecipitation (ChIP) Assay Protocol

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Abstract

Reagents -

Basic cell culture media: respective to the cell line used and experimental design

Distilled water: Invitrogen® UltraPure™ Distilled Water (catalog number 19977015) or equivalent product from other vendor.

Highthroughput (HT) Pro A or G 96 well plate or Spin Column (Chromatrap® HT ChIP-qPCR kit; catalog number 500161 or 500162 or 500163)

96-well collection plate: USA scientific® TempPlate non-skirted 96-well PCR plate natural (catalog number 1402-9596) or equivalent product from other vendor.

QIAquick™ PCR purification kit (Qiagen®; catalog number 28104) or equivalent product from other vendor.

Hypotonic Buffer (Chromatrap® HT ChIP-qPCR kit; catalog number 500161 or 500162 or 500163)

Digestion Buffer (Chromatrap® HT ChIP-qPCR kit; catalog number 500161 or 500162 or 500163)

Lysis Buffer (Chromatrap® HT ChIP-qPCR kit; catalog number 500161 or 500162 or 500163)

Column Conditioning Buffer (Chromatrap® HT ChIP-qPCR kit; catalog number 500161 or 500162

or 500163)

Wash Buffer 1 (Chromatrap® HT ChIP-qPCR kit; catalog number 500161 or 500162 or 500163)

Wash Buffer 2 (Chromatrap® HT ChIP-qPCR kit; catalog number 500161 or 500162 or 500163)

Wash Buffer 3 (Chromatrap® HT ChIP-qPCR kit; catalog number 500161 or 500162 or 500163)

Elution Buffer (Chromatrap® HT ChIP-qPCR kit; catalog number 500161 or 500162 or 500163)

5M NaCl: dissolve 292g of NaCl in 800mL of H_2O. Adjust the volume to 1L with H_2O.

1M $NaHCO_3$: dissolve 12.6g of $NaHCO_3$ in 100 mL of H_2O. Adjust the volume to 150 mL with H_2O.

Protease Inhibitor Cocktail (PIC): Cell signaling Technology® (catalog number 5871) or equivalent product from other vendor.

Proteinase K: Qiagen® (catalog number 19133) or equivalent product from other vendor.

Proteinase K stop solution: 100mM Phenylmethylsulfonyl fluoride (PMSF). Weigh 1.742g PMSF (Sigma-Aldrich®, catalog number 10837091001) and add DMSO (Sigma-Aldrich®, catalog number D2650) to a final volume of 100mL. Dissolve completely.

PBS: HyClone™ Phosphate Buffered Saline (1X) (catalog number SH30256.01) or equivalent product from other vendor.

Formaldehyde: VWR® 37% w/w aqueous solution (catalog number 97064-604) or equivalent product from other vendor.

0.65M Glycine: Dissolve 488g of glycine into 1L of H_2O to make 6.5M glycine (10X). Dilute with PBS to make 1X before each experiment.

Micrococcal Nuclease: Cell Signaling Technologies® (catalog number 10011S) or equivalent product from other vendor.

1.3-1.5% Agarose gel: dissolve 1.3-1.5g of agarose powder into 100mL of TAE buffer by microwave heating. Visualize DNA under UV light by adding appropriate amount of Ethidium Bromide, or use an equivalent method.

Add appropriate amount of Ethidium Bromide to cooled down gel solution to visualize DNA under UV light.

0.5M EDTA: Dissolve 186.1g EDTA into 700mL of H_2O. Adjust the volume to 1L with H_2O.

100bp ladder: NEB® (catalog number N0467S) or equivalent product from other vendor.

Materials -

Heat block

Rotator

Centrifuge

Microcentrifuge

37ºC incubator

Nanodrop

Sonicator

UV light imager

Rocking platform

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Protocol

Chromatin Sample Preparation

Step 1.

Culture between 1-15 million cells. Collect cells by spinning down at 500xg at 4ºC for 5 minutes. Wash cells with PBS at room temperature.

DURATION
00:05:00

Chromatin Sample Preparation

Step 2.

Remove the PBS and add freshly made basic cell culture media (it should not contain any serum or large molecular weight proteins to avoid any interferences from those proteins) containing1% formaldehyde (CH2O) to cross-link the DNA-protein complexes.

Chromatin Sample Preparation

Step 3.

Incubate for 10 minutes at room temperature with gentle agitation on a rocking platform.

DURATION
00:10:00

Chromatin Sample Preparation

Step 4.

Remove the fixation solution by spinning down at 500xg at 4ºC for 5 minutes. To quench there action, for cell count of 1-5 million, add 3mL of 0.65M glycine solution; for cell count of 5-10million, add 4mL of 0.65M glycine solution; for cell count of 10-15 million, add 5mL of 0.65M glycine solution.

DURATION
00:05:00

Chromatin Sample Preparation

Step 5.

Incubate at room temperature for 5 minutes with gentle agitation on a rocking platform.

Chromatin Sample Preparation

Step 6.

Remove the glycine solution by spinning down at 500xg at 4ºC for 5 minutes. Discard thesupernatant. The pellet can be frozen at -80ºC after adding 1µL of Protease Inhibitor Cocktail (PIC).

DURATION
00:05:00

Chromatin Sample Preparation

Step 7.

To lyse the cells, for cell count of 1-5 million, add 0.4mL of Hypotonic Buffer; for cell count of 5-10

million, add 0.8mL of Hypotonic Buffer; for cell count of 10-15 million, add 1mL of HypotonicBuffer to the cell pellet. Resuspend the cells and incubate at 4ºC for 10 minutes.

DURATION
00:10:00

Chromatin Sample Preparation

Step 8.

Centrifuge the hypotonic slurries at 5,000xg at 4ºC for 5 minutes to collect the nuclei.

DURATION
00:05:00

Chromatin Sample Preparation

Step 9.

For cell count of 1-5 million, add 0.3mL of Digestion Buffer; for cell count of 5-10 million, add 0.4mL of Digestion Buffer; for cell count of 10-15 million, add 0.5mL of Digestion Buffer to the nuclei, immediately followed by adding 2µL of PIC to each sample.

Chromatin Sample Preparation

Step 10.

Add Micrococcal Nuclease to each sample to digest the DNA. Mix by inverting the tube severaltimes and incubate at 37ºC for 20 minutes. Mix by inversion every 3-5 minutes. The amount andincubation time of Micrococcal Nuclease required to digest the genomic DNA to an optimal 150900 bp length may need to be determined empirically for individual cell types.

DURATION
00:20:00

Chromatin Sample Preparation

Step 11.

Stop digestion by adding 10µL of 0.5M EDTA per sample and place the sample on ice.

Chromatin Sample Preparation

Step 12.

Pellet nuclei by centrifugation at 12,000xg at 4ºC for 1 minute. Discard the supernatant.

DURATION
00:01:00

Chromatin Sample Preparation

Step 13.

Resuspend nuclear pellet in Lysis Buffer and incubate the sample on ice for 10 minutes to lyse the nuclei. Alternatively, sonicate to shear the DNA. The time and strength for sonication may need to be determined empirically.

DURATION
00:10:00

Chromatin Sample Preparation

Step 14.

Centrifuge the sample at 16,000xg at 4ºC for 10 minutes. Transfer the supernatant to a clean dry microcentrifuge tube.

Chromatin Sample Preparation

Step 15.

Add 1µL of PIC to each sample and mix.

Chromatin Sample Preparation

Step 16.

Chromatin sample is now ready for ChIP Assay. If the sample is not to be used immediately,store at -80ºC. It is recommended that the shearing efficiency is analyzed at this stage to ensurethat 150-900bp fragments are obtained during shearing the DNA to increase the ChIP efficiency.

Addendum: Shearing Efficiency Analysis

Step 17.

Take 50µL aliquot of sheared chromatin from each sample into a new microcentrifuge. Add 5µLof 1M NaHCO3, 5µL of 5M NaCl and 50µL of Distilled Water. Mix thoroughly and incubate at 65ºC (heat block) for two hours or incubation can be extended for overnight.

DURATION

02:00:00

Addendum: Shearing Efficiency Analysis

Step 18.

Remove the sample from heat block, add 1µL of Proteinase K to each sample, vortex briefly and perform a spin down at 500xg at 4ºC for 5 minutes. Incubate at 37ºC for one hour.

DURATION

01:00:00

Addendum: Shearing Efficiency Analysis

Step 19.

Add 2µL of Proteinase K Stop Solution to each sample, vortex briefly and perform a short spindown.

Addendum: Shearing Efficiency Analysis

Step 20.

Purify the DNA by QIAquick™ PCR purification kit, according to manufacturer's manual.

Addendum: Shearing Efficiency Analysis

Step 21.

Quantify the DNA in the sample using a Nanodrop. This will be used to determine the volume ofchromatin to load in ChIP assay.

Addendum: Shearing Efficiency Analysis

Step 22.

To ensure 150-900bp fragments have been obtained during shearing the DNA, run each DNAsample on a 1.3-1.5% agarose gel and visualize against a marker of known size DNA fragments(e.g., 100bp ladder).

Chromatin Immunoprecipitation Assay

Step 23.

Mix the Chromatin Sample, Protease Inhibitor Cocktail (PIC), optimal quantity of BioLegend'sGo-ChIP-GradeTM Purified antibodies and add Column Conditioning Buffer to make final 1mL slurries. Gently rotate at 4ºC for one hour or incubation can also be extended to overnight.

Chromatin Immunoprecipitation Assay

Step 24.
Prepare the high-throughput (HT) Protein A or G 96 well plate or Spin Column by adding 600µLof Column Conditioning Buffer in each well or column and allow it to flow through via gravity (15minutes).

DURATION
00:15:00

Chromatin Immunoprecipitation Assay

Step 25.
Discard the flow-through and repeat steps 2 and 3.

Chromatin Immunoprecipitation Assay

Step 26.

Remove the slurries from the rotator following 4ºC incubation and briefly spin down to remove residual liquid from the caps.

Chromatin Immunoprecipitation Assay

Step 27.
Load the entire 1mL slurries and allow to flow completely through the high-throughput (HT)Protein A or G 96 well plate or Spin Column at room temperature (approximate 15-20 minutes).

DURATION
00:20:00

Chromatin Immunoprecipitation Assay

Step 28.
Add 600µL of Wash Buffer 1 to each well or column and centrifuge at 2000xg for plate, or 4000xgfor column, for one minute at room temperature. Discard the flow through and repeat once.

DURATION
00:01:00

Chromatin Immunoprecipitation Assay

Step 29.

Add 600µL of Wash Buffer 2 to each well or column and centrifuge at 2000xg for plate, or 4000xg for column, for one minute at room temperature. Discard the flow through and repeat once.

DURATION
00:01:00

Chromatin Immunoprecipitation Assay

Step 30.
Add 600µL of Wash Buffer 3 to each well or column and centrifuge at 2000xg for plate, or 4000xgfor column, for one minute at room temperature. Discard the flow through and repeat once.

DURATION
00:01:00

Chromatin Immunoprecipitation Assay

Step 31.

Spin dry at 4,000xg for the plate or 16,000xg for the column for one minute at room temperature to remove any remaining liquid from the membrane of the plate or column. Place a clean 96 well collection plate or a collection tube beneath the plate or column.

DURATION
00:01:00

Chromatin Immunoprecipitation Assay

Step 32.

Add 50µL of Elution Buffer to each well or column. Incubate at room temperature for 15 minutes.

DURATION
00:15:00

Chromatin Immunoprecipitation Assay

Step 33.

Centrifuge the plate or column at 4,000xg for the plate, or 16,000xg for the column, for one minute at room temperature to collect the eluted chromatin-protein complex.

DURATION
00:01:00

Chromatin Immunoprecipitation Assay

Step 34.

To each eluted sample, add 5µL of 1M NaHCO3, 5µL of 5M NaCl and 50µL of Distilled Water. Mixthoroughly and incubate at 65ºC on the heat block for two hours or the incubation time can be extended to overnight.

DURATION
02:00:00

Chromatin Immunoprecipitation Assay

Step 35.

Remove the sample from the heat block, add 1µL of Proteinase K to each sample, vortex briefly andperform a short spin down. Incubate at 37ºC for one hour.

DURATION
01:00:00

Chromatin Immunoprecipitation Assay

Step 36.

Add 2µL of Proteinase K Stop Solution to each sample, vortex briefly and perform a short spin down.

Chromatin Immunoprecipitation Assay

Step 37.

Purify the DNA by QIAquick™ PCR purification kit, according to manufacturer’s manual. Purified DNA is ready for downstream real-time qPCR analysis.