Chromatin immunoprecipitation Version 2

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Abstract

This protocol can be ued for chromatin immunoprecipitation of RNAPII and associated factors, as well as histones. The settings are given for HeLa cells and should be adapted for other cell types.

Citation: Gabriel Sanchez,Rosemary Kiernan Chromatin immunoprecipitation. **protocols.io** dx.doi.org/10.17504/protocols.io.knkcvcw
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Guidelines

The protocol given is suitable for a 15 cm^2 dish of HeLa cells at 80 % confluence per IP. DNA shearing conditions are suitable for HeLa cells and should be tested for other cell types. Antibody concentrations used for IP should also be tested for the specific antibodies to be used. Use molecular biology grade reagents and water.

Before start

Prepare the following buffers and store at +4°C:

10 % Sodium deoxycholate:
10 g /100 ml water

Lysis buffer:	**Stock**	**Final Conc**	**Vol (for 2 ml)**
Tris-HCl, pH 7.5	1 M	50 mM	100 µl
KCl	2 M	150 mM	150 µl
EDTA	0.5 M	5 mM	20 µl
NP40 (Igepal)	10 %	1 %	200 µl
SDS	10 %	0.1 %	20 µl
Water			1370 µl
Just before use add:			
Protease inhibitor cocktail	50 x	1 x	40 µl
NaF	1 M	50 mM	100 µl

RIPA Buffer (Stock Solution):	**Stock**	**Final Conc**	**Vol (for 30 ml)**
Tris-HCl, pH 7.5	1 M	50 mM	1.5 ml
KCl	3 M	150 mM	1.5 ml
NP40 (Igepal)	10 %	1 %	3 ml
Water			24 ml

RIPA Buffer:	**Stock**	**Final Conc**	**Vol (for 3 ml)**
RIPA Buffer (Stock Solution)			2.7 ml
for bead washing:			
Water OR			300 µl OR
for IPs:			
Protease inhibitor cocktail	50 x	1 x	60 µl
NaF	1 M	50 mM	150 µl
water			90 µl
(add just before use)			

Resuspension Buffer:	**Stock**	**Final Conc**	**Vol (for 1 ml)**
Tris-HCl, pH 7.5	1 M	50 mM	50 µl
EDTA	0.5 M	5 mM	50 µl
DTT	100 mM	10 mM	100 µl
SDS	10 %	1 %	100 µl
Water			750 µl

RIPA Wash Buffer:	**Stock**	**Final Conc**	**Vol (for 100 ml)**
Tris-HCl, pH 7.5	1 M	50 mM	5 ml
KCl	2 M	150 mM	7.5 ml
NP40 (Igepal)	10 %	0.1 %	1 ml
Sodium deoxycholate	10 %	0.25 %	2.5 ml
Water			84 ml

TE 1 x Wash Buffer	**Stock**	**Final Conc**	**Vol (for 50 ml)**
Tris-HCl, pH 7	1 M	10 mM	500 µl
EDTA	0.5 M	1 mM	100 µl
Water			49.4 ml

Materials

protein A/G plus agarose sc-2003 by Santa Cruz Biotechnology

Complete protease inhibitor cocktail 05052489001 by Roche

normal goat IgG sc-2028 by Santa Cruz Biotechnology

Protocol

Prepare Protein A/G Beads

Step 1.

(Hint: when pipetting beads, cut off ends off of P200 pipette tips to allow the beads to pass through)

For each ChIP, use 160 µl protein A/G agarose beads (equivalent to 40 µl dry bead volume)

Centrifuge beads for 1 min at 6000 rpm

Remove supernatant and add an equal dry bead volume of RIPA buffer (solution for beads) and mix well

Centrifuge 1 min at 2600 rpm, then 10000 rpm for 6 sec

Remove supernatant

Wash 4 times as above with 1 ml RIPA buffer (solution for beads)

Remove supernatant

Add an equal dry bead volume of RIPA buffer (solution for beads), yeast tRNA (200 µg/ml final conc.) and salmon sperm DNA (40 µg/ml final conc.)

Incubate 1 h at room temp on a rotating stand

Centrifuge 1 min at 2600 rpm, then 10000 rpm for 6 sec

Remove supernatant and add 1 dry bead volume RIPA buffer (solution for beads)

Remove 40 µl dry beads using cut-off pipette tip and mix with 160 µl RIPA buffer (solution for beads)

(Keep the remaining bead slurry for pre-clearing of chromatin at step 3)

Add 3 µg of specific antibody or IgG (optimal amount should be determined for each antibody)

Incubate tubes on a rotating stand at +4°C for 4 h

Prepare chromatin extract

Step 2.

Perform cross link by adding 1% formaldehyde (final conc.) directly to cell culture medium

Incubate 10 min at RT

Block with glycine pH7.5 (250 mM final conc.) for 5 min at RT

Wash twice with 10 ml PBS

Scrape cells in 1 ml PBS, split into 2 tubes and centrifuge at 3000 rpm for 5 min

Remove supernatant

(Note: it is possible to flash freeze cell pellets in liquid N2 at this stage)

Resuspend each pellet in 600 µl RIPA lysis buffer, on ice

Shear DNA in a Bioruptor nano (Diagenode) by 8 cycles, high setting, 30 sec ON, 30 sec OFF (conditions to be determined for other cell types)

Add 450 µl RIPA buffer (solution for IP) to each tube

Centrifuge at 15000 rpm for 10 min at +4°C to remove cell debris

Pool chromatin extracts

(do not freeze at this stage, proceed directly to step 3)

Immunoprecipitation

Step 3.

Pre-clear chromatin extract from step 2 with bead slurry saved from step 1 together with 3 µg normal goat IgG, for 1 h at 4°C

Centrifuge at 10000 rpm for 1 min

Collect supernatant (pre-cleared chromatin extract), remove 100 µl for input, and add the remainder to beads/antibody pre-mix from step 1.

Place tubes on a rotating stand overnight at 4°C.

Washes and Elution

Step 4.

Wash beads 4 times in 1 ml RIPA wash buffer (spin 30 sec at 2500 rpm, then a few seconds at 10,000 rpm to compact the beads, discard supernatant)

Wash once in TE wash buffer

Resuspend beads in 100 µl RIPA resuspension buffer

Reverse cross-link samples at 65°C overnight (IP and input) with agitation (thermomixer)

Spin tubes at 5000 rpm for 1 min and recover supernatant (discard beads)

Add 1 µg Proteinase K to samples and incubate at 45°C for 1 h

Purify DNA from samples using QIAquick PCR purification kit

Elute DNA in 50 µl water