User Guide

Catalog No. CHP2

CHP2 Experienced User Protocol

sigma-aldrich.com/life-science/epigenetics/chip2.html

CHP2 Experienced User Protocol

This protocol has been written for the experienced **CHP2 kit** user who has read & successfully performed ChIPs following the procedure as described in the CHP2 kit user guide. This abbreviated procedure is written for the advanced (repeat) user who has all the required buffers reconstituted & is familiar with the pre-requisites for each section.

Index:
O/N - overnight
H - hour(s)
RT - room temperature
min - minute(s)
PIC – protease inhibitor cocktail

1. Blocking ChIP Next Gen Seq Sepharose®:

- ❑ Rehydrate each vial of ChIP Next Gen Seq Sepharose with 200 µL of water & transfer 100 µL to 0.5 mL tube, store remaining 100 µL ChIP Next Gen Seq Sepharose at 4 °C & use within 1 month
- ❑ Add 5 µL BSA (**A7638**, 20 mg/mL), 1 µL 0.1 M AEBSF & 10 µL salmon sperm DNA (omit DNA for ChIP-Seq)
- ❑ Rotate end-over-end O/N at 4 °C (or 2 H minimum at RT)
- ❑ Transfer to 1.5 mL tube
- ❑ Spin 14K RCF at 4 °C, 3 min
- ❑ Discard supernatant, resuspend pellet with 1 mL Dialysis buffer & spin 14K RCF at 4 °C, 3 min
- ❑ Discard supernatant & repeat wash as above
- ❑ Resuspend pellet in 100 µL Dialysis buffer & add 1 µL 0.1 M AEBSF
- ❑ Store at 4 °C & use within 2 weeks

2. Cross-Link Cells:

- ❑ Grow healthy cell cultures in 500 cm^2 dishes
- ❑ Remove media, rinse cells with 35 mL HEPES Buffered Salt Solution
- ❑ Add 9 mL Trypsin-EDTA, incubate 37 °C, 5 min, dislodge attached cells
- ❑ Add 31 mL complete medium / 500 cm^2 dish & resuspend cells, transfer to 50 mL tube
- ❑ Add 1.2 mL 37 % Formaldehyde/ 40 mL cell suspension
- ❑ Rotate end-over-end exactly 10 min at RT
- ❑ Add 4.1 mL cold 1.25 M Glycine/ 40 mL cell suspension
- ❑ Rotate end-over-end exactly 5 min at RT
- ❑ Spin 200 RCF at 4 °C, 10 min
- ❑ Discard supernatant, resuspend pellet with 50 mL cold 1× Phosphate Buffered Saline
- ❑ Spin 200 RCF at 4 °C, 10 min
- ❑ Prepare chromatin or snap freeze cell pellet in liquid N_2 & store at -80 °C

Complete Cell Lysis Buffer:	
Cell Lysis Buffer	0.9 mL
+ 10 % IGEPAL	0.1 mL
+ PIC (P8340)	10 µL
+ 0.1 M AEBSF	10 µL
Total	**1 mL**

Complete Nuclei Lysis Buffer:	
Nuclei Lysis Buffer	0.5 mL
+ PIC (P8340)	5 µL
+ 0.1 M AEBSF	5 µL
Total	**0.5 mL**

3. Chromatin Prep:

- ❑ Resuspend cross-linked cells in fresh Complete Cell Lysis Buffer (1 mL/ 50 × 10^6 cells)
- ❑ Chill on ice, 20 min
- ❑ Dounce 1 mL aliquots in 2 mL glass Dounce homogenizer with 15 strokes of B pestle, transfer lysate to 1.5 mL tubes
- ❑ Spin 2.5 K RCF at 4 °C, 5 min
- ❑ Discard supernatant, resuspend nuclei pellet in fresh Complete Nuclei Lysis Buffer (0.5 mL/ 50 x 10^6 cells)
- ❑ Chill on ice, 10 min
- ❑ Sonicate nuclear lysate using pre-determined optimum conditions for each cell line & instrument. (For BioRuptor: Transfer to 15 mL polystyrene tubes, 30s ON + 30s OFF, 3 × 10 min for ChIP-q-PCR & 4-5 × 10 min for ChIP-Seq experiments)
- ❑ Transfer to 1.5 mL tubes
- ❑ Spin 14 K RCF at 4 °C, 10 min
- ❑ Transfer supernatant to new 1.5 mL tube(s) & aliquot
- ❑ Proceed for ChIP or snap freeze in liquid N_2 & store at -80 °C

4. Pre-clearing:

- ❑ Pre-clear the required amount of chromatin with blocked ChIP Next Gen Seq Sepharose, 10 µL per 10^7 cells
- ❑ Rotate end-over-end exactly 15 min at 4 °C
- ❑ Spin 14 K RCF at 4 °C, 5 min
- ❑ Transfer supernatant (pre-cleared chromatin) to new 1.5 mL tube(s), 125 µL/ tube

IP Dilution Buffer:	
IP Dilution Buffer	1 mL
+ PIC (P8340)	10 µL
+ 0.1 M AEBSF	10 µL
Total	**1 mL**

5. Ab Binding:

- ❑ Remove 12.5 µL chromatin for "10 % Input" & keep aside on ice
- ❑ Dilute 125 µL chromatin with 375 µL IP Dilution buffer (+ protease inhibitors) + Ab (specific/control)
  - For positive control ChIP: Add 0.5 µL Mu IgG (**I5381**)
  - For negative control ChIP: Add 1 µL RNAP II Ab (**WH0005430M1**)
- ❑ Rotate end-over-end O/N at 4 °C (or for abundant target rotate end-over-end for 2 H at 4 °C)

6. Bridging Ab & IP:

- ❑ Add 1 µL Rb anti-Mouse Ab (**M7023**) for ChIPs with monoclonal antibodies (or appropriate bridging antibody)
- ❑ Rotate end-over-end 1 H at 4°C
- ❑ Add 10 µL blocked ChIP Next Gen Seq Sepharose (for chromatin from > 2 x10^6 cells) or 5 µL blocked ChIP Next Gen Seq Sepharose (for chromatin from ≤ 2 x10^6 cells)
- ❑ Transfer to new 1.5 mL tube
- ❑ Spin 14K RCF at 4°C, 4 min

Dialysis Wash Buffer:	
Dialysis Wash Buffer	1 mL
+ 0.1 M AEBSF	10 µL
Total	**1 mL**

IP Wash Buffer:	
IP Wash Buffer	1 mL
+ 0.1 M AEBSF	10 µL
Total	**1 mL**

IP Elution buffer:	
Water	425 µL
+ 10 % SDS	50 µL
+ 1 M NaHCO3	25 µL
Total	**500 µL**

7. Washings:

❑ Discard supernatant, resuspend pellet with fresh 1 mL Dialysis buffer (+ AEBSF), spin 14 K RCF at 4 °C, 3 min

❑ Discard supernatant & repeat wash as above

❑ Discard supernatant, resuspend pellet with fresh 1 mL IP Wash buffer (+ AEBSF), spin 14 K RCF at 4 °C, 3 min

❑ Discard supernatant & repeat wash three more times as above

❑ Remove last traces of IP Wash buffer with a quick spin 14 K RCF at 4 °C, 1 min

8. ChIP Elution:

❑ Add 50 µL fresh IP Elution buffer to each ChIP Next Gen Seq Sepharose pellet

❑ Incubate RT 15 min with vortexing on moderate setting (3 on Vortex Genie)

❑ Spin 14K RCF at RT, 3 min

❑ Transfer supernatant to new 1.5 mL tube

❑ Repeat Elution: Add 50 µL fresh IP Elution buffer to each ChIP Next Gen Seq Sepharose pellet

❑ Incubate RT 15 min with vortexing on moderate setting (3 on Vortex Genie)

❑ Spin 14K RCF at RT, 3 min

❑ Transfer & pool supernatants in same 1.5 mL tube

9. Cross-Link Reversal:

❑ Spin 14K RCF at RT, 5 min to remove residual ChIP Next Gen Seq Sepharose

❑ Transfer supernatant to new 1.5 mL tube

❑ To "10 % Input" sample add 87.5 µL IP Elution buffer & 1 µL Proteinase K (P4850) & incubate 65 °C, 1 H

❑ Add 12 µL 5 M NaCl to all samples (ChIPs & Input)

❑ Rotate end-over-end O/N at 67 °C (or boil for 15 min for ChIP-q-PCR only)

10. DNA Purification:

- ❑ Chill on ice 5 min, thaw by hand until clear
- ❑ Add 1µL RNAse A
- ❑ Incubate 37 °C, 15 min
- ❑ Assemble one set of DNA purification column in collection tube for each ChIP & Input sample
- ❑ Add 500 µL DNA Binding buffer to each prep, vortex, transfer to column
- ❑ Spin 12K RCF at RT, 1 min
- ❑ Discard flow through, wash column with 200 µL DNA Wash buffer
- ❑ Spin 12K RCF at RT, 1 min
- ❑ Discard flow through, wash column again with 200 µL DNA Wash buffer
- ❑ Spin 12K RCF at RT, 1 min
- ❑ Transfer column to fresh 1.5 mL tube
- ❑ Add 30 µL DNA Elution buffer for ChIP-q-PCR (or add 6 µL Elution buffer for ChIP-chip or ChIP-Seq)
- ❑ Spin 12K RCF at RT, 1 min
- ❑ Use purified DNA for PCR, q-PCR, WGA, labeling, quantitation or sequencing

11. Q-PCR Assay:

❑ q-PCR setup for Actin & ZNF-333-3' (per reaction):

9.5 µL	Water
1.0 µL	5 µM Primers mix
12.5 µL	2X PCR Ready-mix (component of **S4438**)
2.0 µL	ChIP'ed DNA or 1 % Input or water
25 µL	Total volume

❑ Thermocycling conditions:

Denature	95 °C	2 min
40 cycles of	95 °C	30 sec
	60 °C	30 sec
	72 °C	1 min, read

❑ Use the ChIP Q-PCR Data analysis excel shell (**http://www.sigmaaldrich.com/life-science/epigenetics/chip2.html**) to calculate ChIP yield i.e. "**% Input**" & "**Fold Enrichment**" with respect to non-specific Ab

For more information, visit
sigma-aldrich.com/life-science/epigenetics/chip2.html

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