Dec 26, 2025

Chromatin immunoprecipitation (ChIP) in 22Rv1 cells

DOI
https://dx.doi.org/10.17504/protocols.io.eq2ly5dzevx9/v1

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Protocol Citation: Xin Xu, Yujuan Wang 2025. Chromatin immunoprecipitation (ChIP) in 22Rv1 cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2ly5dzevx9/v1

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Protocol status: Working
We use this protocol and it's working

Created: December 21, 2025

Last Modified: December 26, 2025

Protocol Integer ID: 235595

Keywords: chromatin immunoprecipitation, ChIP, ChIP-seq, epigenetics, transcription regulation, histone modification, protein-DNA interaction, cancer cells, chromatin biology, dna purification, cells this protocol, chip, cell, chd9 knockdown, purification, dna

Disclaimer

This protocol is provided for research use only and may require optimization depending on experimental conditions.

Abstract

This protocol describes chromatin immunoprecipitation (ChIP) in 22Rv1 cells with or without CHD9 knockdown, followed by DNA purification and ChIP-seq library preparation.

Materials

Cells

- 22Rv1 cells (control and CHD9 knockdown)
- 5 × 10^6 cells per ChIP reaction

Reagents

- Formaldehyde (to 1% final concentration)
- Glycine (to 125 mM final concentration)
- PBS (cold)

Modified RIPA buffer (pH 8)

- 10 mM Tris-HCl, pH 8
- 1 mM EDTA
- 140 mM NaCl
- 1% Triton X-100
- 0.1% SDS
- 0.1% sodium deoxycholate
- Protease inhibitor (add fresh)

High-salt modified RIPA buffer

- Same as modified RIPA buffer, with NaCl adjusted to 500 mM

LiCl wash buffer (pH 8)

- 10 mM Tris-HCl, pH 8
- 1 mM EDTA
- 250 mM LiCl
- 0.5% NP-40
- 0.1% sodium deoxycholate

TE buffer

- 10 mM Tris-HCl, pH 8
- 1 mM EDTA

Decrosslinking buffer

- 1% SDS
- 0.1 M NaHCO3

Antibodies

- Anti-CHD9 (Proteintech, 13402-1-AP)
- Anti-H3K27ac (Abcam, ab4729)

Beads

- Protein A magnetic beads (Thermo Fisher, 10002D)
- Protein G magnetic beads (Thermo Fisher, 10004D)

Enzymes and kits

- RNase A (Thermo Fisher, EN0531)
- Proteinase K (Thermo Fisher, AM2546)
- PCR purification kit (QIAGEN, 28004)
- ThruPLEX DNA-seq kit (Takara, R400676)

Equipment

- Bioruptor sonicator (Diagenode)
- End-over-end rotator (4°C)
- Thermomixer or shaking incubator
- Magnetic rack or centrifuge
- Microcentrifuge

Before start

- All buffers should be freshly prepared or thawed on ice.
- Add protease inhibitor to lysis buffers immediately before use.
- Perform all steps on ice or at 4°C unless otherwise indicated.
- Pre-chill centrifuges, rotators, and sonicator to 4°C.
- Use low-retention, DNA-free tubes and tips.

Crosslinking

20m

1 Harvest 5 × 10^6 22Rv1 cells per sample.

2 Add formaldehyde to a final concentration of 1%, and incubate for 10 min at room temperature. 10m

3 Quench crosslinking by adding glycine to a final concentration of 125 mM. 5m

4 Pellet cells and wash twice with cold PBS, and then discard supernatant completely. 5m

Cell lysis and chromatin shearing and pre-cleaning

1h 30m

5 Resuspend cell pellet in 300 µL modified RIPA buffer supplemented with protease inhibitor.

6 Sonicate chromatin using a Bioruptor (Diagenode) at 4°C:
18 cycles
30 s ON / 30 s OFF 30m

7 NOTE: Sonication conditions may require optimization depending on cell density and instrument performance.

8 Briefly centrifuge to remove insoluble debris and transfer supernatant to a new tube.

9 Add **40 µL protein A/G beads** to the sonicated chromatin. Incubate with rotation at **4°C**. Transfer the pre-cleared chromatin to a new tube. 1h *

Immunoprecipitation

18h 30m

10 For each ChIP, prepare antibody-coated beads: 6h
- **5 µg antibody**
- **11 µL Protein A beads**
- **11 µL Protein G beads**

Incubate with rotation at **4°C for at least 6 h**.

11 Combine pre-cleared chromatin with antibody-coated beads. Incubate **overnight at 4°C** with rotation. 12h

12 Wash beads at **4°C**, rotating for **5 min** per wash: 30m
- **Once** with **0.5 mL modified RIPA buffer**
- **Once** with **0.5 mL high-salt modified RIPA buffer (500 mM NaCl)**
- **Once** with **0.5 mL LiCl buffer**
- **Twice** with **0.5 mL TE buffer (pH 8)**

Between washes, collect beads and carefully remove supernatant.

Elution and decrosslinking 13h

13 Resuspend beads in 100 µL decrosslinking buffer. Add 1 µL RNase A. Incubate at 37°C for 30 min with shaking. 30m

14 Add 2 µL Proteinase K. Incubate at 55°C for 30 min with shaking. 30m

15 Reverse crosslinks by incubating at 65°C overnight with shaking. 12h

DNA purification and library preparation 2h 30m

16 Purify DNA using the **QIAGEN PCR purification kit (28004)** according to the manufacturer's instructions. Elute purified ChIP DNA in nuclease-free water or elution buffer. 30m

17 Use purified DNA for **Illumina ChIP-seq library construction** with the **ThruPLEX DNA-seq kit (Takara, R400676)** following the manufacturer's protocol. 2h