CHIP-Adembeads and ChIP-Adem-Kit Protein A/G

Quick Protocol

INSTRUCTIONS MANUAL FOR USE WITH 04240, 04241, 04340, 04341

CHROMATIN IMMUNOPRECIPITATION

Before starting please read the entire protocol, you must optimize sonications conditions to shear your DNA. Sheared chromatin to an average lenght of about 200-800 bp is recommended.

Products

- Blocking Buffer
- Ip Buffer
- Specific or non relevant ChIP antibodies
- Sheared cross-linked Chromatin
- Washing Buffer
- Elution Buffer
- Proteinase K (10mg/ml)

Equipment

- Magnet
- Microtubes
- Rotators, thermomixer

Procedure

1- ChIP-Adembeads Protein A/G Blocking Step

- Transfer **25µl of ChIP-Adembeads** to a microtubube 15ml.
- Add **225µl of Blocking Buffer.**

Blocking Buffer must be stored at room temperature (RT).

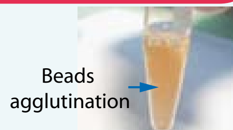
- Homogenize by pipeting and **incubate 15 min at RT** under mixing.

2-Immunoprecipitation

- Place the tube on the magnet until supernatant clearing and discard the supernantant.
- Resuspend the beads in **125µl of IP Buffer.**
- Add **1-3 µg of antibody** (specific or non relevant) and **incubate 1h at RT.**

At this step, beads agglutination may occur corresponding to antibody capture by the beads.

- Add **900µl of IP Buffer** and resuspend the beads by pipeting.
- Add **100µl of sheared cross- linked chromatin.**
- **Incubate overnight at 4°C under agitation.**



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CHROMATIN IMMUNOPRECIPITATION

Procedure

3-Washing steps

- Place the tube on the magnet until supernatant clearing and discard the supernatant.
- Resuspend the beads in **300µl of Washing Buffer I** and **incubate 10 min at RT** under mixing.
- Place the tube on the magnet until supernatant clearing and discard the supernatant.
- Resuspend the beads in **300µl de Washing Buffer II** and **incubate 10 min at RT** under mixing.
- Place the tube on the magnet until supernatant clearing and discard the supernatant.
- Resuspend the beads in **300µl de Washing Buffer III** and **incubate 10 min at RT** under mixing.
- Place the tube on the magnet until supernatant clearing and discard the supernatant.
- Resuspend the beads in **300µl de Washing Buffer IV**.
- Place the tube on the magnet until supernatant clearing and discard the supernatant.
- Resuspend the beads in **300µl de Washing Buffer IV.**

At this step, resuspend thoroughly by pipeting the magnetic particles.

4- Elution and Reversion

- Place the tube on the magnet until supernatant clearing and discard the supernatant.
- Resuspend thoroughly by pipeting the beads in **300µl of Elution Buffer.**
- Add **10µl of Proteinase K (10mg/ml)** and **incubate 2h at 37°C** under agitation.
- Place the tube on the magnet until supernatant clearing and discard the supernatant.
- **Collect the supernatant** in a new microtube and **incubate the supernatant overnight at 65°C** under mixing.

Do not perform the incubation at 65°C in the presence of beads.

Tips

If you use ChIP-Adembeads, we recommend NaCl Buffer for washing steps with increasing concentrations (150-500nM) and SDS for Elution BUffer.

If you use ChIP-Adem-Kit, the Elution Buffer contains SDS. Before PCR analysis, purify your DNA.

For one ChIP with 25 µl of ChIP-Adembeads, 3 µg of antibody is the optimal amount.

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