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TECH TIPS FOR SUCCESSFUL ChIP EXPERIMENTS

New to ChIP or just want to improve?
Our tech tips will help you achieve successful ChIP experiments.

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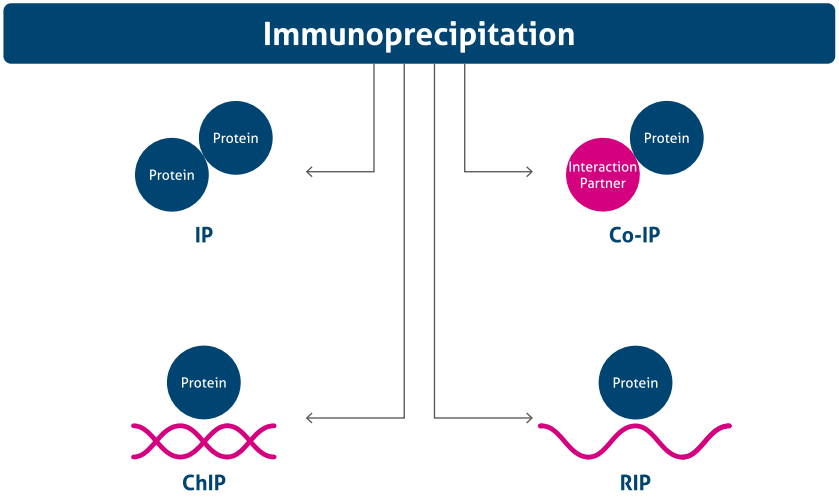
IMMUNOPRECIPITATION TYPES

There are several types of immunoprecipitation:

- IP used to isolate a single protein
- Co-Immunoprecipitation (Co-IP)
- Chromatin Immunoprecipitation (ChIP)
- RNA Immunoprecipitation (RIP)



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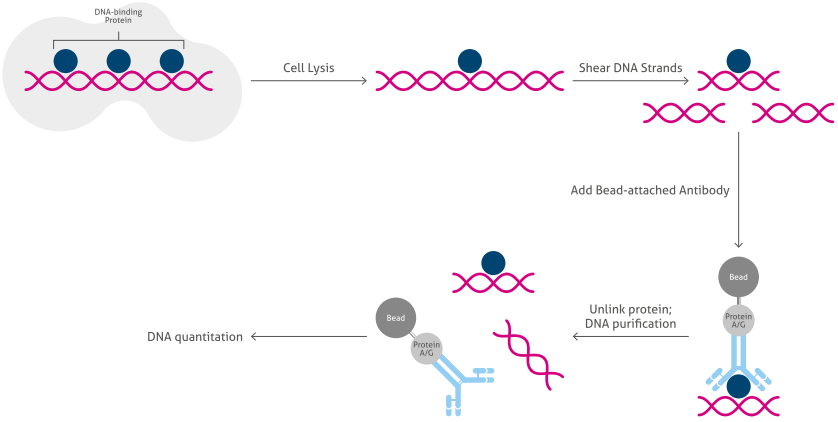
ChIP OVERVIEW

- **ChIP provides a live cell picture of the native chromatin structure and factors bound to genes in different functional states.**
- **ChIP methodology involves protein –DNA cross-linking.**
- **Isolated, crude chromatin is sonicated to small fragments, usually with an average size of 300–1000 bp.**
- **Protein-specific antibodies are used to immunoprecipitate fragments of chromatin.**



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TECH TIPS FOR ChIP EXPERIMENTS

ChIP Tip 1: **Always keep cells/tissue on ice**

- **Temperature is critical. Perform cell lysis at 4°C. Keep the samples ice-cold and use ice-cold buffers.**

ChIP Tip 2: **Under/over cross-linking**

Please note: When using paraformaldehyde, ensure that it is freshly prepared (final concentration of 1%–1.5%).

- **Under cross-linking can prevent the disassociation of protein–DNA complexes and result in poor yield.**
- **Over cross-linking can mask epitope sites crucial for antibody binding, prevent chromatin shearing, and inhibit the successful uncross-linking of the protein–DNA complex.**

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TECH TIPS FOR ChIP EXPERIMENTS

ChIP Tip 3: **Chromatin shearing and sonication**

- Avoid large fragments in the tissue suspension.
- Pipette with cut tips for better homogenization.
- Ensure the sonicator probe is not in contact with the tube wall.
- Increase the number of sonication steps; however, avoid increasing the time (or the power) of each step as this may overheat the sample and lead to loss of antigenicity.
- Add ice to the sonicator to avoid the sample overheating.

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TECH TIPS FOR ChIP EXPERIMENTS

ChIP Tip 4:

Bead and primary antibody choice (Beads)

- Always fully resuspend beads by vortexing before pipetting.
- Always store at 4°C and never allow beads to dry out.
- Check the subclass of your antibody is compatible with Protein A/G.

Affinity of human immunoglobulins to Protein A and G

The full table of human, mouse, rat and guinea pig immunoglobins can be found in our technical guide "Overview & Technical Tips: Immunoprecipitation".

*To download click **here***

Species	Subclass	Protein A	Protein G
Human	IgA	Variable	–
	IgD	+	–
	IgE	+	–
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	–	++++
	IgG_4	++++	++++
	IgM	Variable	–

Binding Capacity:

++++	***Strong Binding***
+++/++	***Medium Binding***
Variable/–	***Weak or No Binding***

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TECH TIPS FOR ChIP EXPERIMENTS

ChIP Tip 4:

Bead and primary antibody choice (Antibody)

- Verify your antibody of interest is ChIP validated.
- Insufficient amount of antibody can result in not enough material for successful PCR analysis.
- Too much antibody can increase PCR background.

Negative ChIP controls

- Use non-immune IgG in the IP incubation mix from the same species the antibodies were produced in.
- Incubate IP fraction with beads (without antibody coating).

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TECH TIPS FOR ChIP EXPERIMENTS

ChIP Tip 5: **IP efficiency in reverse cross-linking IP**

- Usually, a 15-minute incubation at 95°C is sufficient. Some samples require Proteinase K treatment for four or more hours at 65°C.

ChIP Tip 6: **DNA elution and purification**

- Use different washing buffers (low & high salt, LiCl nd TE buffers).
- While using a commercial purification column, check the column is completely dry after the wash step as any leftover moisture will inhibit elution.
- Make sure the elution buffer is placed directly onto the silica membrane and allowed to adsorb for at least one minute.

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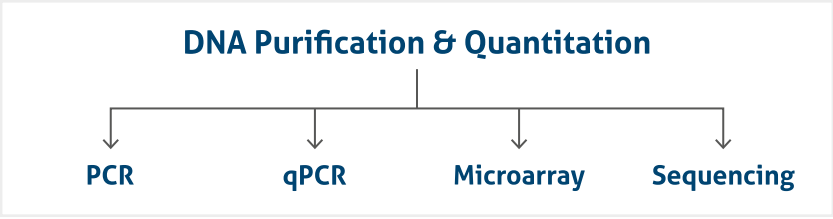
TECH TIPS FOR ChIP EXPERIMENTS

ChIP Tip 7:

Analysis on Immunoprecipitated DNA

- To avoid variations between replicates, add the same amount of protein G/A-agarose or magnetic beads for all samples. Ensure beads are well suspended while pipetting.
- Complete the elution of chromatin from protein G/A beads. Elution is optimal at 65°C with frequent mixing to keep beads suspended in solution (~10 minutes).



Please note: A weak PCR signal or no DNA amplification shown in the samples may be due to an inadequate primer result in the PCR amplified region spanning the nucleosome-free region.

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