Instruction Manual

Plant ChIP-seq kit

Cat. No. **C01010150**

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diagenode

Content

Kit Materials

The content of the kit is sufficient to perform 24 ChIP assays, 6 chromatin extractions and 6 chromatin shearing optimization reactions. Store the components at the indicated temperature upon receipt (Table 1).

Table 1: Components supplied with the Plant ChIP-Seq kit

Description	Quantity	Storage
10x Crosslinking Buffer	13 ml	4°C
Extraction Buffer 1	130 ml	4°C
Extraction Buffer 2	160 ml	4°C
Extraction Buffer 3	35 ml	4°C
10x ChIP Dilution Buffer	13 ml	4°C
Sonication Buffer	2.2 ml	4°C
Protein-A coated magnetic beads	530 µl	4°C **Do NOT freeze!**
Wash Buffer 1	50 ml	4°C
Wash Buffer 2	50 ml	4°C
Wash Buffer 3	50 ml	4°C
Wash Buffer 4	26 ml	4°C
Elution Buffer 1	16 ml	4°C, incubate at RT before use
Elution Buffer 2	640 µl	4°C
DNA Precipitant	1.6 ml	4°C
DNA Co-precipitant	200 µl	**-20°C**
Glycine	14 ml	**4°C**
Rabbit IgG	10 µg/10 µl	**-20°C**
ChIP-seq grade premium antibody H3K4me3	10 µg/10 µl	**-20°C**
FLC-ATG Primer pair (positive control)	5 µM each/50 µl	**-20°C**
FLC-Intron1 Primer pair (negative control)	5 µM each/50 µl	**-20°C**

Required Materials Not Provided

- Formaldehyde (high-quality methanol-free)
- Miracloth
- Funnels
- Vacuum pump
- Desiccator
- Liquid nitrogen
- dH_2O
- Ceramic mortar and pestle
- 50 ml tubes
- 1.5 ml Safelock tubes
- 1.5 ml Bioruptor® tubes (Depending on the Bioruptor® model used)
- Bioruptor®
- Thermomixer
- Rotating wheel
- Magnetic rack
- Phenol/Chloroform/Isoamylalcohol {25:24:1}
- Chloroform/Isoamylalcohol {24:1}
- ß-Mercaptoethanol
- Protease Inhibitor Cocktail for plants (e.g. Protease inhibitor cocktail for plant cell and tissue extracts, Sigma Aldrich #P9599)

Remarks before starting

1. Starting material

This protocol has been optimized for chromatin extraction from 1 g fresh weight (fw) of *Arabidopsis thaliana* seedlings. One ChIP reaction is performed with 50 µl of the yielded chromatin equalling approximately 0.2 g (fw) of seedlings. It is possible to use more material per IP, however we recommend performing separate IPs and pooling the IP'd DNA before purification.

2. Shearing optimization and sheared chromatin analysis

Before starting the ChIP, the chromatin should be sheared to fragments ranging between 200 and 600 bp. Our kits and protocols are optimized for chromatin shearing using the Bioruptor® (Bioruptor® Pico (#B01060001); Bioruptor® Plus (#B01020001); Bioruptor® Standard (#B01010001)) in combination with a water cooling system (#B02010003; 115V or #B02010002; 230V). If you use a different sonicator, the sonication conditions must be optimized accordingly. Tubes and corresponding volumes depend on the Bioruptor® system used, refer to www.diagenode.com. We recommend using 1.5 ml Bioruptor® Microtubes with Caps (#C30010016) for the Bioruptor® Pico and 1.5 ml TPX microtubes (#C30010010) for the Bioruptor® Standard and Plus. The shearing conditions mentioned in the protocol are adequate for chromatin from different plant tissues. However we recommend optimizing the sonication conditions for each tissue before processing a large number of samples. Perform an initial sonication time course experiment to evaluate the extent of chromatin fragmentation. A protocol to assess the shearing efficiency can be found in the "Additional Protocols" section.

3. Magnetic beads

This kit includes DiaMag protein A-coated magnetic beads (#C03010020). Make sure the beads do not dry during the procedure as this will result in reduced performance. Keep the beads in a homogenous suspension at all times when pipetting. Variation in the amount of beads will lead to lower reproducibility. Do not freeze the beads. The amount of beads needed per IP depends on the amount of antibody used for the IP. The protocol below uses 20 µl of beads. The binding capacity of this amount is approximately 5 µg of antibody. With most of Diagenode's high quality ChIP-seq grade antibodies the recommended amount to use is 1 to 2 µg per IP reaction. However, if you plan to use more than 5 µg of antibody per IP we recommend increasing the amount of beads accordingly.

4. Negative and positive IP controls

The kit contains a negative (IgG, #C15410206) and a positive (**H3K4me3**, #C15410003) control antibody. We recommend including one IgG negative IP control in each series of ChIP reactions. We also recommend using the positive control ChIP-seq grade **H3K4me3** antibody at least once. The kit also contains qPCR primer pairs for amplification of a positive and negative control target for **H3K4me3** in Arabidopsis thaliana (**FLC-ATG** and **FLC-Intron1**, respectively).

5. Preparation of buffers

Check **Elution buffer 1** for precipitation upon storage. If necessary, warm to 37°C until the precipitate has fully dissolved. **Crosslinking Buffer** and **ChIP Dilution Buffer** are provided as 10x concentrates. Before use prepare the necessary amount of 1x Buffer using dH20. Proteinase Inhibitor Cocktail and ß-mercaptoethanol should be added freshly, where stated.

Short protocol: Plant Chromatin Immunoprecipitation

STEP 1. Crosslinking of plant tissue

1. Prepare the necessary amount of complete 1x **Crosslinking Buffer** and supplement it with 1% Formaldehyde, keep on ice.
2. Harvest 1 g plant material into a 50 ml tube and keep the harvested tissue cooled on ice.
3. Add 20 ml complete 1x **Crosslinking Buffer** (containing formaldehyde) to each tube and submerge the tissue in Buffer.
4. Place the opened 50 ml tube (on ice) in a desiccator and apply vacuum for 15 min. If the tissue is floating on top of the Buffer slowly release the vacuum after 5 min, submerge the tissue and re-apply the vacuum for the remaining 10 min. Release the vacuum slowly.
5. Add 2 ml **Glycine** solution (provided) to each tube. Mix the solution and apply vacuum for additional 5 min. Release the vacuum slowly.
6. Discard the **Crosslinking Buffer** and wash the plant tissue 2 times with 40 ml of deionized water (4 °C; not supplied).
7. Remove the plant tissue from the tube and dry it thoroughly using paper towels. Insert the plant tissue into new 50 ml tubes, close the tubes and snap-freeze them in liquid nitrogen. Store the tubes at -80 °C or proceed directly with chromatin extraction.

STEP 2. Chromatin extraction from plant tissue and chromatin shearing

8. Prepare the necessary amounts of complete Extraction Buffers 1-3 by adding ß-mercaptoethanol and Protease Inhibitor Cocktail and keep them cold.
9. Grind the plant tissue thoroughly to a fine powder with ceramic mortar and pestle using liquid nitrogen to keep the tissue cold at all times.
10. Transfer the powder to a pre-cooled 50 ml tube and add 20 ml of cold complete **Extraction Buffer 1**. Vortex vigorously until the tissue is completely homogenized in the Buffer.
11. Filter the suspension twice through Miracloth and collect the flow-through in a fresh 50 ml tube on ice.
12. Centrifuge for 20 min at 1000 g at 4 °C.
13. Wash the pellet containing the nuclei 5 times with 5 ml complete **Extraction Buffer 2**. In each washing step carefully resuspend the pellet by gentle shaking (no vortexing or pipetting). Centrifuge at for 10 min 1000 g at 4°C after each wash.
14. Wash once with 5 ml **Extraction Buffer 3** as in the previous step.
15. Resuspend the pellet in 300 µl of **Sonication Buffer** (supplemented with Protease Inhibitor Cocktail) by pipetting and transfer the suspension to a 1.5 ml sonication tube. If the volume is bigger than 300 µl, split it in two sonication tubes.
16. Sonicate the chromatin with the Bioruptor® for 8-10 cycles with the settings 30 sec ON / 30 sec OFF at 4 °C.
17. Transfer the sonicated chromatin to a standard 1.5 ml tube and centrifuge for 10 min at 12.000 g at 4 °C.
18. Transfer the supernatant to a new 1.5 ml tube, this is the extracted chromatin. Use 50 µl to check the shearing efficiency on an agarose gel (see Additional Protocols section). Store the chromatin at -80 °C or proceed directly with immunoprecipitation.

STEP 3. Magnetic Immunoprecipitation

19. Determine the total number of IP samples in the experiment. Prepare the necessary amount of 1x **ChIP dilution Buffer** and supplement it with Protease Inhibitor Cocktail, keep on ice.
20. Transfer the necessary amount of DiaMag Protein A-coated magnetic beads for all IPs (20 µl per IP) to a fresh 1.5 ml tube for washing.
21. Wash the beads 4 times with 1 ml of ice-cold 1x **ChIP Dilution Buffer**.
22. After the last wash, resuspend the beads in the original volume (+10 µl) with 1x **ChIP Dilution Buffer**. Use one fresh tube per antibody and add the necessary amount of washed beads (20 µl of per IP). If the final volume is less than 100 µl fill up to this volume with 1x **ChIP Dilution Buffer**.
23. Add x µl of antibody or control IgG (using the same amount as the test antibody) to the beads and incubate on a rotating wheel overnight at 4°C (or at least 4 hours).
24. Wash the antibody-coated beads 3 times with 1 ml 1x **ChIP Dilution Buffer** and after the last wash discard the supernatant and resuspend the beads in the original volume (+10 µl).
25. Per IP: Dilute 50 µl of the sheared chromatin in a ratio of 1:5 by adding 200 µl of cold 1x **ChIP Dilution Buffer** and mix thoroughly. Place 2.5 µl of the diluted chromatin aside (-20 °C) to be used later as INPUT.
26. Prepare the ChIP reaction mixes in 1.5 ml tubes as follows:
    - 20 µl **antibody** coated magnetic beads
    - 250 µl **diluted chromatin** (1:5)
    - 1x **Plant Protease Inhibitor Cocktail**
27. Incubate the tubes for 4 h or overnight on a rotating wheel (40 rpm) at 4 °C.
28. Briefly spin the tubes and place them in the magnetic rack. Wait for one minute and remove the supernatant. Wash the beads 2 times with 1 ml **Wash Buffer 1** by gently shaking the tubes to resuspend the beads and incubating for 5 min at 4 °C on a rotating wheel. Spin the tubes shortly and place them in the magnetic rack. Wait for one minute and remove the supernatant.
29. Repeat the washing step as described above with **Wash Buffer 2** and **Wash Buffer 3**, performing 2 washes with each.
30. Perform one washing step with 1 ml **Wash Buffer 4** per sample.

STEP 4. Elution, De-crosslinking and DNA isolation

31. After removing the last Buffer, add 400 µl of **Elution Buffer 1** to the beads and incubate for 30 min in a thermomixer at 65 °C (shaking at 1300 rpm).
32. Briefly spin the tubes and place them in the magnetic rack. Transfer the supernatant to a new tube.
33. Add 16 µl of **Elution Buffer 2** to the isolated supernatant and incubate for at least 4 h (or overnight) in a thermomixer at 65 °C (1300 rpm). Also add 397.5 µl **Elution Buffer 1** and 16 µl of **Elution Buffer 2** to the INPUT sample(s) kept aside at Step 3 point 25 and treat it like the IP samples from this step onwards.

DNA purification

34. Add an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) to each sample. Vortex and centrifuge for 3-5 min at 12 000 g at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
35. Add 1 volume of chloroform/isoamyl alcohol (24:1). Vortex and centrifuge for 3-5 min at 12 000 g at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
36. Precipitate the DNA by adding 40 µl of **DNA Precipitant**, 5 µl of **DNA Co-precipitant** and 1 ml of cold 100 % ethanol to the sample. Incubate for 30 min at -80 °C (or for 2 h at -20 °C). Centrifuge for 25 min at 12 000 g at 4 °C.
37. Carefully remove the supernatant and add 500 µl of ice-cold 70 % ethanol to the pellet. Centrifuge for 10 min at

12 000 g at 4 °C.

38. Carefully remove the supernatant and air dry the pellet at RT until all remaining ethanol has evaporated.
39. Resuspend the pellet in 20 µl of **dH2O** (DNase-free).
40. For PCR, dilute the DNA 1:10 and use 5 µl per reaction.

Quantitative PCR analysis

41. Prepare the qPCR master mix (20 µl PCR master mix + 5 µl DNA sample = total volume 25 µl) as follows:
    - 12.5 µl of a 2x SYBR Green PCR master mix
    - 1 µl of primer mix (5 pmol of each primer)
    - 6.5 µl DNase-free water
42. As template use 5 µl of purified diluted IP'd DNA sample and purified diluted INPUT(s).
43. Perform the PCR using the following conditions: 3 to 10 minutes denaturation step at 95°C (according to the manufacturer's recommendations), followed by 45 cycles of 30 seconds at 95 °C, 30 seconds at 60 °C and 30 seconds at 72 °C, followed by an elongation step for 10 min at 72 °C. These conditions may require optimisation depending on the type of SYBR Master Mix or qPCR system used.

Detailed protocol

STEP 1. Crosslinking of plant tissue

Caution: Steps 1-7 should be carried out under the fume hood!

1. Prepare 1x **Crosslinking Buffer** (20 ml per 1 g fresh weight plant tissue) by diluting 2 ml of **10x Crosslinking buffer** with 18 ml dH20. Add formaldehyde (high-quality methanol-free solution) to a final concentration of 1 % and store it at 4 °C.
2. Harvest 1 g of plant material into a 50 ml tube and keep harvested tissue cooled on ice.
3. Add 20 ml of 1x Crosslinking Buffer supplemented with 1% formaldehyde (4°C) to each 50 ml tube containing 1 g of plant material. Make sure that the tissue is submerged in buffer and not floating on top of the buffer.
4. Crosslink the plant tissue in the (opened) 50 ml tube by placing the tube in a desiccator and applying vacuum for 15 min (on ice). If the tissue is floating on top of the buffer slowly release the vacuum after 5 min, submerge the tissue and reapply the vacuum for the remaining 10 min. Release the vacuum slowly.
5. Stop the crosslinking by adding 2 ml of **Glycine** solution (provided) to each 50 ml tube containing 1 g of plant tissue. Mix the solution and apply vacuum for additional 5 min. Release the vacuum slowly.
6. Discard the Crosslinking Buffer and wash the plant tissue twice with 40 ml of deionized water (4 °C; not supplied). For washing add the water to the 50 ml tube, invert the closed tube twice and discard the water. Alternatively you can use a funnel and Miracloth to wash the plants with adequate amount of deionized water.
7. Remove the plant tissue from the 50 ml tube and dry it thoroughly using paper towels. Insert the plant tissue into new 50 ml tubes, close the tubes and snap-freeze them in liquid nitrogen. At this point the tissue can be stored at -80 °C or be directly used for the chromatin extraction.

STEP 2. Chromatin extraction from plant tissue and chromatin shearing

Caution: Steps 8-14 should be carried out under the fume hood!

Description	Reagent	Concentration / Dilution
Extraction Buffers 1-3	ß-Mercaptoethanol	Add to a final concentration of 5 mM
	Plant Protease Inhibitor Cocktail	Add according to manufacturer's instructions
Sonication Buffer	Plant Protease Inhibitor Cocktail	Add according to manufacturer's instructions

8. Prepare the necessary amounts of complete **Extraction Buffers 1-3** by adding ß-mercaptoethanol and Protease Inhibitor Cocktail and keep them cold.
9. Fill a ceramic mortar with liquid nitrogen and add the plant tissue. Grind the plant tissue thoroughly using mortar and pestle to a fine powder in liquid nitrogen being careful not to let the tissue thaw at any time.
10. Transfer the powder to a pre-cooled 50 ml tube and add 20 ml of cold **Extraction Buffer 1**. (If you transfer the powder in liquid nitrogen, make sure it has evaporated completely before adding **Extraction Buffer 1**.) Vortex vigorously until the tissue is completely homogenized in the buffer.
11. Filter the suspension twice through Miracloth (Calbiochem) and collect the flow-through in a fresh 50 ml tube on ice.
12. Centrifuge for 20 min at 1000 g at 4 °C.
13. Wash the pellet containing the nuclei 5 times with 5 ml **Extraction Buffer 2**. In each washing step carefully resuspend the pellet by gentle shaking (no vortexing or pipetting). Centrifuge at for 10 min 1000 g at 4 °C after each wash.

14. Wash once with 5 ml **Extraction Buffer 3** as in point 13.Resuspend the crude nuclear pellet in 300 µl of **Sonication Buffer** by pipetting and transfer the suspension to a 1.5 ml sonication tube (tubes depend on the Bioruptor® model used). Since the **Sonication Buffer** contains SDS, be careful not to cause bubbles by pipetting. If the volume is bigger than 300 µl, split it in two sonication tubes.
15. Sonicate the chromatin with the Bioruptor® for 8-10 cycles with the settings 30 sec ON / 30 sec OFF at 4 °C. Optimization may be required depending on the tissue type, fixation time and Bioruptor® model used. We recommend performing a time course experiment in order to specify the optimal sonication conditions.
16. Transfer the sonicated chromatin to a standard 1.5 ml tube and centrifuge for 10 min at 12.000 g at 4 °C.
17. Transfer the supernatant to a new 1.5 ml tube, this is the extracted chromatin. Purify the DNA from 50 µl of the chromatin to check the shearing efficiency on an agarose gel (see Additional Protocols section).

At this point the chromatin can be stored at -80 °C or directly used for the immunoprecipitation. Avoid multiple freeze/thaw cycles.

STEP 3. Magnetic immunoprecipitation

Before starting with step 3, prepare the necessary amount of 1x ChIP Dilution buffer and add the indicated reagents

Buffer	Reagent	Concentration / Dilution
1x ChIP Dilution Buffer	Plant Protease Inhibitor Cocktail	Add according to manufacturer's instructions

18. The chromatin extraction from 1 g of plant material yields 300 µl of sheared plant chromatin. This protocol has been optimized for 50 µl plant chromatin per IP reaction. Although it is possible to use more chromatin, we recommend performing separate IP reactions and pooling the samples together prior to DNA purification.
19. Determine the total number of IPs in the experiment. Please note that we recommend including one negative control in each experiment (IP with the IgG negative control). Transfer the necessary amount of DiaMag Protein A-coated magnetic beads (#C03010020) for all IPs (20 µl per IP) to a fresh 1.5 ml tube for washing.
20. Wash the beads by adding 1 ml of ice-cold 1x **ChIP Dilution Buffer**, resuspend by pipetting up and down several times and place the tubes in the DiaMag1.5-magnetic rack (#B04000003). Wait for one minute to allow the beads to be captured by the magnet and remove the supernatant by pipetting. Repeat the washing step 3 more times. Alternatively, you can centrifuge the tubes for 5 minutes at 1.300 g, discard the supernatant and keep the bead pellet.
21. After the last wash, resuspend the beads in the original volume (+10 µl) with 1x **ChIP Dilution Buffer**. Use one fresh tube per antibody and add the washed beads (20 µl of per IP) that will be used with this antibody. If the final volume is less than 100 µl fill up the volume with 1x **ChIP Dilution Buffer**.
22. Add x µl of antibody to the beads and incubate on a rotating wheel overnight at 4°C (or at least 4 hours). The quantity of antibody depends on its quality. The quantity of IgG should be relative to that of the antibody. If you use the anti-**H3K4me3** antibody provided here, use 1 µg antibody per IP and the same amount of IgG for the negative control IP.
23. Wash the antibody coated beads (as in point 21) 3 times with 1 ml 1x **ChIP Dilution Buffer** and after the last wash discard the supernatant and resuspend the beads in the original volume (+10 µl).
24. Per IP: Dilute 50 µl of the sheared plant chromatin in a ratio of 1:5 by adding 200 µl of cold 1x **ChIP Dilution Buffer** and mix thoroughly. Scale according to the number of IPs performed with the same chromatin. Place 2.5 µl of the diluted chromatin aside (-20 °C) to be used as INPUT later.
25. Prepare the ChIP reaction mixes in 1.5 ml tubes as follows:
    - 20 µl antibody coated magnetic beads
    - 250 µl diluted chromatin (1:5)
    - 1x Plant Protease Inhibitor Cocktail

26. Incubate the tubes for 4 h on a rotating wheel (40 rpm) at 4 °C. (Alternatively this incubation can be performed overnight.)
27. After the incubation briefly spin the tubes and place them in the DiaMag1.5-magnetic rack. Wait for one minute and remove the supernatant. Wash the beads twice with **Wash Buffer 1**. To wash the beads, add 1 ml of **Wash Buffer 1**, gently shake the tubes to resuspend the beads and incubate for 5 min at 4 °C on a rotating wheel. Spin the tubes shortly and place them in the DiaMag1.5-magnetic rack. Wait for one minute and remove the supernatant.
28. Repeat the washing step as described above with **Wash Buffer 2** and **Wash Buffer 3**. Perform 2 washes with each.
29. Perform one washing step with 1 ml **Wash Buffer 4** per sample.

STEP 4. Elution, De-crosslinking and DNA isolation

Caution: Steps 34-36 should be carried out under the fume hood!

31. After removing the last buffer, add 400 µl of **Elution Buffer 1** to the beads and incubate for 30 min in a thermomixer at 65 °C (shaking at 1300 rpm, or alternatively mix tubes every 10 min)
32. Briefly spin the tubes and place them in the DiaMag1.5-magnetic rack. Transfer the supernatant to a new tube.
33. Add 16 µl of **Elution Buffer 2** and incubate for at least 4 h (or overnight) in a thermomixer at 65 °C (1300 rpm). Also add 397.5 µl **Elution Buffer 1** and 16 µl of **Elution Buffer 2** to the INPUT sample(s) kept aside at step 3 point 25 and treat the INPUT sample like the IP samples from this step onwards.

DNA purification with Phenol Chloroform extraction:

34. Add an equal volume of phenol/chloroform/isoamyl alcohol {25:24:1} to each sample. Vortex and centrifuge for 3-5 min at 12 000 g at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
35. Add 1 volume of chloroform/isoamyl alcohol {24:1}. Vortex and centrifuge for 3-5 min at 12 000 g at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
36. Precipitate the DNA by adding 40 µl of **DNA precipitant**, 5 µl of **DNA co-precipitant** and 1 ml of cold 100 % ethanol to the sample.
37. Incubate for 30 min at -80 °C (or for 2 h at -20 °C). Centrifuge for 25 min at 12 000 g at 4 °C.
38. Carefully remove the supernatant and add 500 µl of ice-cold 70 % ethanol to the pellet. Centrifuge for 10 min at 12 000 g at 4 °C.
39. Carefully remove the supernatant and air dry the pellet at RT until all remaining ethanol has evaporated.
40. Resuspend the pellet in 20 µl of dH2O (DNase-free).
41. For PCR, dilute the DNA 1:10 and use 5 µl per reaction.

qPCR analysis and results

We recommend analysing the IP'd DNA by qPCR using at least 1 positive and 1 negative control target.

1. Prepare the qPCR master mix (total volume 25 µl = 20 µl PCR master mix + 5 µl DNA sample):
   - 1 x SYBR Green PCR master mix (e.g.: iQ SYBR Green supermix)
   - 5 pmol of each primer (1 µl if you use the provided primer pair mixes)
   - fill up to 20 µl with DNase-free water
2. As template use 5 µl of purified diluted IP'd DNA sample and purified diluted INPUT(s).
3. Perform the PCR using the following conditions: 3 to 10 minutes denaturation step at 95°C (please check carefully the manufacturer's recommendations about Taq polymerase activation time), followed by 45 cycles of 30 seconds at 95 °C, 30 seconds at 60 °C and 30 seconds at 72 °C, followed by an elongation step for 10 min at 72 °C. These conditions may require optimisation depending on the type of Master Mix or qPCR system used.

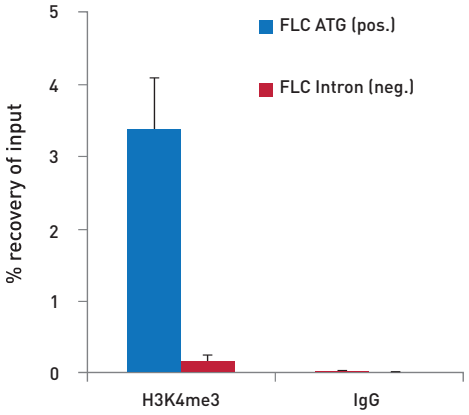

Figure 1: ChIP was performed on *Arabidopsis thaliana* (Col-0) seedlings 14 days after germination (dag) using our premium anti-H3K4me3 ChIP-seq grade antibody (#C15410003) and rabbit IgG (#C15410206). Data shown is derived from biological replicates. Sheared chromatin from 0.2 g (fw) of seedlings, 1 µg of H3K4me3 antibody and 1 µg of the negative IgG control were used per IP. Quantitative PCR was performed with the positive control FLC-ATG and the negative control FLC-Intron1 primer sets from the kit. The recovery is expressed as % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

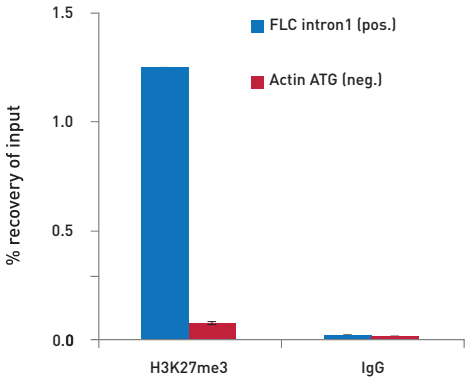

Figure 2: ChIP was performed on *Arabidopsis thaliana* (Col-0) seedlings 14 days after germination (dag) using our premium anti-H3K27me3 ChIP-seq grade antibody (#C15410195) and rabbit IgG (#C15410206). Data shown is derived from biological replicates. Sheared chromatin from 0.2 g (fw) of seedlings, 2 µg of H3K27me3 antibody and 2 µg of the negative IgG control were used per IP. Quantitative PCR was performed with primer pairs for FLC-Intron1 (positive control) and Actin ATG (negative control). The recovery is expressed as % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

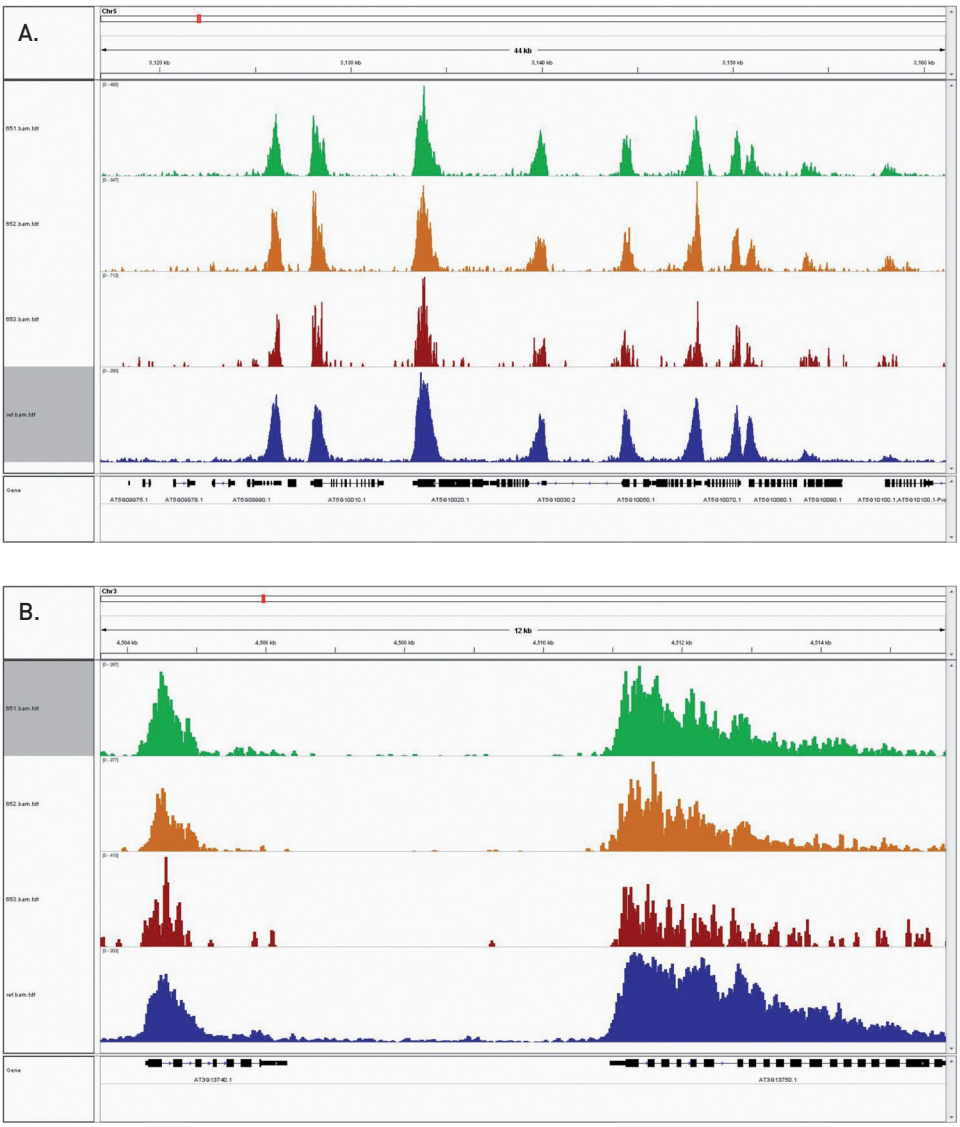

Figure 3: ChIP-seq was performed on *Arabidopsis thaliana* (Col-0) seedlings (grown for 14 days) using our premium anti-H3K4me3 ChIP-seq grade antibody (#C15410003). Libraries were prepared with our MicroPlex Library Preparation™ kit (#C05010010) from 1 ng (green), 500 pg (orange) and 100 pg (red) IP'd DNA and sequenced on an Illumina® HiSeq 2500. The enrichment in blue represents a public dataset (NCBI GEO Dataset GSM1193621) that we used as an external reference. **A)** Enrichments along a wide region of chromosome 5 are uniform regardless of the starting material amount. **B)** Detailed view of a region of chromosome 3 illustrating the similarity in the enrichment structures.

Additional protocols

Sheared chromatin analysis

Reagents not supplied:

- RNase (e.g. Ambion, #AM 2286 A)
- Phenol/chloroform/isoamyl alcohol {25:24:1}
- Chloroform/isoamyl alcohol {24:1}
- 100 % Ethanol
- 70 % Ethanol
- Agarose and TAE buffer
- DNA ladder marker
- DNA loading dye

Caution: Steps 4-6 should be carried out under the fume hood!

1. Use the 50 µl aliquot of sheared chromatin from Step 2 point 18 to analyse the shearing efficiency.
2. Treat the chromatin with an RNase according to its manufacturer's instructions. (Usually using 1 µl RNase cocktail and incubation 1 h at 37 °C)
3. Add 350 µl of **Elution Buffer 1** and 16 µl **Elution Buffer 2** to the chromatin sample. Mix thoroughly and incubate the samples for 4 h (or overnight) at 65°C in a thermoshaker (1300 rpm).
4. Add an equal volume of phenol/chloroform/isoamyl alcohol {25:24:1} to the sample, vortex and centrifuge for 3-5 min at 12 000 g at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
5. Add an equal volume of chloroform/isoamyl alcohol {24:1} to the sample, vortex and centrifuge for 3-5 min at 12 000 g at RT. Transfer the top aqueous phase into a new 1.5 ml tube.
6. Precipitate the DNA by adding 1/10 volume of **DNA Precipitant**, 5 µl of **DNA Co-precipitant** and 1 ml of cold 100 % ethanol to the sample. Incubate for 30 min at -80 °C (or for 2 h at -20°C). Centrifuge for 25 min at 12,000 g at 4°C.
7. Carefully remove the supernatant and add 500 µl of ice-cold 70 % ethanol to the pellet. Centrifuge for 10 min at 12 000 g at 4°C.
8. Carefully remove the supernatant and air dry the pellet at RT until all remaining ethanol has evaporated.
9. Resuspend the pellet in 20 µl of dH2O which corresponds to the purified DNA from the sheared chromatin.
10. Add DNA loading dye (according to manufacturer's instructions) and analyse the sample(s) on a 1.5 % agarose gel along with DNA size marker to visualize the shearing efficiency.

Troubleshooting guide

Process	Protocol step	Issues and resolutions
Cross-linking (formaldehyde fixation)	Optimize crosslinking time	Poor cross-linking causes DNA loss, elevated background, and/or reduced antigen availability in chromatin. Empirically determine optimal cross-linking time for maximal specificity and efficiency of ChIP. The optimal duration of cross-linking varies between cell type and protein of interest. Short cross-linking time (5-10 minutes) may improve shearing efficiency. Cross-linking duration should not exceed 15 minutes or shearing will be inefficient.
	Optimize formaldehyde concentration	Low formaldehyde concentrations like 1% (weight/volume) may improve shearing efficiency. For some proteins, however, especially those that do not directly bind DNA, this might reduce cross-linking efficiency and thus the yield of precipitated chromatin. Empirically determine the formaldehyde concentration as some antigen epitopes may be more sensitive to formaldehyde.
	Quality of formaldehyde	We recommend to use fresh, high-quality, methanol-free formaldehyde solution.
	Quenching of the cross-linking reaction	Use a final concentration of 125 mM glycine to stop the cross-linking reaction
Cell lysis	Make sure cells disrupt completely	Do not use too many cells per amount of lysis buffer (w/v) in order to reach complete cell disruption. Follow the instructions in the protocol.
	Maintain cold temperature during lysis	It is critical to perform the cell lysis at 4°C (cold room) or on ice. Keep the samples on ice at all times and use cold buffers.
	Prevent protein degradation	The amount of cells required per IP reaction is determined by cell type, protein of interest and the quality of antibodies used. Follow the advice given in the manual.
Number of cells required	Determine the number of cells per IP	The amount of cells required per IP reaction is determined by cell type, protein of interest and the quality of antibodies used. Follow the advice given in the manual.
Chromatin shearing	Optimization of shearing conditions	Shearing conditions need to be optimized empirically for each cell type, cell number and fixation time used. For optimization, a time course experiment is highly recommended and the chromatin shearing optimization modules can be used (C01020010; C01020011; C01020012)
	Analysis of sheared chromatin	Purify the DNA from the sheared chromatin as described in the protocol in order to analyze the shearing. We strongly advise to perform the reverse cross-linking step before the analysis. DNA from ≥100.000 cells can be visualized on a 1-1.5% agarose gel.
	Sample temperature	Maintain the temperature of the samples at 4°C before, during and after the shearing in order to maintain sample integrity.
	Optimize SDS concentration in shearing buffer	A high percentage of SDS favours better sonication but inhibits immunoselection. Optimize the SDS concentration (0.05% to 1%) depending on the cell type and cell number used
	Dilution of sheared chromatin prior to ChIP	For the immunoprecipitation step the SDS concentration needs to be reduced to 0.1% to 0.2%. (e.g. if the shearing buffer contains 1% SDS, dilute the chromatin 1:10 with the buffer used in the IP reaction)
Sonication with the Bioruptor®	Bioruptor® models	Sonication conditions differ depending on the Bioruptor® model used. Refer to the Bioruptor® manuals and protocols available on www.diagenode.com for specific instructions.
	Sonication conditions	We strongly advice to perform a time course experiment to assess shearing efficiency for the specific sample used. Sonicate chromatin samples for 1 to 3 runs of 5 to 10 cycles: (30 sec ON / 30 sec OFF) with high power setting. Precool the water bath to 4°C and make sure to maintain the temperature during sonication.
	Bioruptor® accessories	For optimal shearing results use tubes and chromatin volumes as advised in the Bioruptor® manuals and protocols available on www.diagenode.com.
	Chromatin shearing with Diagenode modules tips	You can also use the LowCell ChIP kit for shearing. 25 µl of complete Buffer B are added per 10 000 cells. After 5 minutes lysis on ice, 75 µl of HBSS are added and chromatin is sheared in 100 µl aliquots. Sheared chromatin have to be diluted 2 times with the complete ChIP Buffer tC1 from the True MicroChIP kit (Protocol STEP3 point 21) before adding antibodies.
	Water cooler	Temperature of the sonication bath can be controlled using the Diagenode water cooling system (B02010003; B02010002)

Magnetic beads	Keep beads in suspension	Beads should not dry out at any time.
	Storage	Store magnetic beads at 4°C. Do not freeze.
	Handling of magnetic beads	Use the DiaMag 0.2 or DiaMag 1.5 magnetic racks for the separation of beads and buffer, e.g. during the washing steps.
	Centrifugation methods	Use gentle centrifugation in order to pellet the beads (up to 1500 g)
Protease inhibitors	Storage	Store provided P.I. mix at -20°C and thaw directly before use.
	Add to buffers directly before use	Add protease inhibitor mix to buffers directly before use. Since some inhibitors are unstable in solution, use buffer within 24 h.
Antibodies	Antibody binding capacity to protein A or protein G	Some inhibitors are unstable in solution. The provided P.I. mix should be kept frozen at -20°C and thawed before use.
	Antibody quality	Add protease inhibitor mix to buffers, just before use, in HBSS (Steps 1 and 2), Lysis Buffer tC1 (Step 2), ChIP Buffer tC1 (Step 3). Discard within 24 hours.
	Determine amount of antibody per IP	Add phosphatase inhibitors or others to Lysis Buffer tC1 and ChIP Buffer tC1, if necessary, depending on your research field and protein(s) of interest. Add NaBu for histone ChIPs.
	Antibody-antigen recognition may affect ChIP efficiency	Antibody-antigen recognition can be significantly affected by cross-linking resulting in loss of epitope accessibility and/or recognition.
Negative ChIP controls	IgG control	Use the non-immune IgG fraction from the same species the antibodies were produced in as a negative control.
	No-antibody control	Do not add antibody to the IP to serve as a negative control. Alternatively incubation with uncoated beads could also be used as a negative ChIP control.
	Usage of un-blocked and blocked antibodies	Use one antibody in ChIP and the same antibody that is blocked with specific peptide. To specifically block one antibody, pre-incubate the antibody with saturating amounts specific peptide for its epitope for about 30 minutes at room temperature before using it in the IP incubation mix.
	Determine number of negative controls needed	If multiple antibodies of the same species are to be used with the same chromatin preparation then a single negative ChIP control is sufficient for all of the antibodies used.
Immunoprecipitation conditions	Temperature	We advise to perform the IP reaction on a rotating wheel at 4°C, and for duration of 2 to 16 hours depending on the antibody used.
	Ultrasonic water bath	The IP reaction can be performed in an ultrasonic bath in order to accelerate antigen binding to antibodies, which is the rate limiting step in ChP.
qPCR	Primer design	Primer length: 18 to 24 nucleotides and primer Tm: 60°C (+/-3.0°C)/ % GC: 50% (+/-4%)
	Include negative and positive controls	Negative PCR controls: PCR with DNA from samples IP'd with non-immune antibodies (negative IgG). Alternatively, PCR using DNA from ChIP samples and primers specific for a DNA region to which your antigen of interest is not binding. Positive PCR control: PCR using input DNA.
	Troubleshoot high Ct values	Use more input chromatin in the case of high Ct values.
	Determine the ratio between Ct(NegCtrl) and Ct(Target)	The ratio between target IP and negative control IP depends on the antibody used.
	Minimize background	Keep the antibody binding beads in suspension during the experiment. Check by eye that equal pellets of beads are present in each tube. Washes are critical.
	Analyse PCR products with melting curve	The melting curve analysis tells you if only the desired PRC product has been amplified. If there are several products the Ct values may be misleading.
Sample storage	Samples can be frozen at several steps of the protocol	Snap freeze the samples in liquid nitrogen (e.g. fixed cell pellets or sheared chromatin).Pellets of formaldehyde fixed cells can be stored at -80°C for at least one year. Sheared chromatin can be stored at -80°C for months, depending on the protein of interest. Purified DNA from ChIP and input samples can be stored at -20°C for months. Thaw samples on ice before use and avoid multiple freeze/thaw cycles.

Ordering information

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NOTICE TO PURCHASER

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