## Product Information

M-Fast® Chromatin immunoprecipitation Kit
Catalog Number: m-CHIP-03 (16 Assays) Stored at 2-8 ${ }^{\circ} \mathbf{C}$

## Description:

This kit provides an innovative technology and a fast, simple procedure for rapid immunoprecipitation of targeted Protein-DNA interaction complex from small amount of cells/tissues (down to 1,000 cells), to identify the special proteins associated with the DNA regions (chromatin), including the specific modified histones, transcription factors or co-factors.

## Kit contains:

| Components (provided in the kit) | Components (prepared by user) |
| :--- | :--- |
| immobilizing buffer (2 vials) <br> dissolved in 2.0 mL distilled water prior to use | Interested antibodies (0.2-1ug) <br> Positive/negative control antibodies (0.2ug) |
| Micron-well strip (16 wells) with adhesive sealing films | $37 \%$ Formaldehyde solution (300 ul) |
| Nuclei isolation buffer (300ul) | 1 M Glycine solution (1.2mL) |
| IP Buffer (10ml) contained protease inhibitors | Primers for qPCR assays |
| $1 \times$ washing buffer ( 25 ml$)$ | PCR/qPCR reagents |
| DNA isolation reagent-A $(2.0 \mathrm{ml}), \mathrm{B}(200 \mathrm{ul}), \mathrm{C}(400 \mathrm{ul})$ | $1 \times P B S / T B S$ |
| DNA precipitation solution $(2.0 \mathrm{~mL})$ | Gel analysis reagents/qPCR machine |
| DNA spin column (20 unites) | $260 / 280 \mathrm{~nm}$ spectrometer |

Protocol: (Keep all buffers and cell/tissue samples on ice)

## 1. Coating interested antibodies into the pre-treated micro-wells:

- Add 100 ul of immobilizing buffer and 2 ul of the interested antibody ( $0.2-2 \mathrm{ug}$ ) or normal rabbit IgG (Negative Control) or Anti-RNA Polymerase II(Positive Control) into the microwells. Mix thoroughly by pipette up and down several times. (Recommended Antibody Dilutions: 1:50)
- Cover the wells with adhesive sealing film or Parafilm and incubate at $4^{\circ} \mathrm{C}$ for overnight or at room temperature for 1-1.5 hours. Aspirate liquids by taping the micron-wells on absorbent paper towel.
- Wash wells 3 times with 200 ul washing buffer (1X). Aspirate liquids by taping the micronwells on absorbent paper towel.
- Add 100 ul IP buffer into the wells. Incubate for 30-60 minutes at room temperature with gentle shaking. The wells are ready to perform IP assays at the step 5 (Immunoprecipitation).
Note, if using protein A/G beads or other co-IP beads, see the technical highlights (Prepare the micronwells coating with Protein A/G beads)

2. Cross-link protein-DNA and Quenching:(performing this step under ventilation hoods)

- Add 15 ul of $37 \%$ Formaldehyde solution directly into the cell culture tube/dish/microplate well containing 500ul media (approximately $1-10 \times 10 \mathrm{e} 5 \mathrm{cells} / 5-20 \mathrm{mg}$ tissues), and incubate for 8 minutes at room temperature with gentle shaking.

Note, if working on frozen cells/tissues or trypsinized cells, spin down the cells/tissues. Discard the supernatant and resuspend the cells/tissues with 500 ul PBS. Then, add 15 ul of $37 \%$ formaldehyde and incubate for 15 minutes at room temperature with gentle shaking.

- Add 62 ul of ice cold 1 M glycine solution and incubate for 5 minutes at room temperature with gentle shaking. Aspirate liquids.

3. Isolate Nuclei from cells/tissues:

- Wash cells/tissues once with 250 ul ice-cold washing buffer. Aspirate liquids.
- Add 250 ul IP buffer and 15 ul Nuclei isolation buffer into the cells/tissues and incubate on ice for 2 minutes. Pipette up and down several times and transfer all suspension into a sonication

[^0] Convenience, Speed, Safety and Economy.
tube. Note, if working on tissue samples, stroke tissues for 15-20 strokes with a pre-chilled Teflon pestle homogenizer for releasing the nuclei.

- Vortex vigorously for 15 seconds and centrifuge at 5,000 xg for 5 minutes at $4^{\circ} \mathrm{C}$. Discard the supernatant.
- Add 200 ul IP buffer to resuspend the nuclei pellet and ready for sonication homogenization.

4. Sonication homogenization:

- Using a pre-washed, clean sonicator, at 20\% power speed for 15-20 seconds, repeated 10 times. Keep tubes on ice and wait 30 seconds between each pulse to avoid over-heat the samples during sonication. (Note: optimize the shearing conditions to obtain 200-1000bp DNA fragments and avoid foaming formation or over-sheared DNA fragments.)
- Centrifuge the sonicated samples at $10,000 \mathrm{xg}$ for 10 minutes at $4^{\circ} \mathrm{C}$. Discard pellet.
- Transfer all the supernatant into a new clean tube. Save 10-20ul for DNA input control and perform the DNA isolation with step 6. (The supernatant can be stored at $-80^{\circ} \mathrm{C}$.)

5. Immunoprecipitation (IP):

- Divide the sonicated supernatant into 3-4 micron-wells containing 100ul IP buffer, prepared from step 1.

Set up the IP assays as following:

| 1) add $45-60$ ul supernatant into the micron-well coating with the interested antibody |
| :--- |
| 2) add 45-60ul supernatant into the micron-well coating with positive control, |
| 3) and 45-60ul supernatant into the micron-well coating with negative control. |

- Cover the wells with adhesive sealing film or parafilm and incubate on a rocking platform for $1-1.5$ hours at room temperature or $37^{\circ} \mathrm{C}$ incubator with gentle shaking. Aspirate liquids by taping the micron-wells on absorbent paper towel.
- Wash wells 3 times with 200 ul washing buffer and aspirate liquids by taping the micron-wells on absorbent paper towel. (The remained unbound proteins and non-specific DNA fractions were washed out).

6. DNA isolation and Cross-link reversal:

- Add 100 ul DNA isolation reagent-A and 10 ul DNA isolation reagent-B into the Micron-wells or the tube of DNA input control. Cover the wells/tube with adhesive sealing film or Parafilm and incubate at $50-60^{\circ} \mathrm{C}$ in the water bath or hybridization oven for 15 minutes. Mix thoroughly by pipette up and down several times every 5 minutes.
- Add 20ul DNA isolation reagent-C into the Micron-wells and mix thoroughly by pipette up and down several times. Transfer the suspension into a clean 1.5 ml tube.
- Incubate at $95^{\circ} \mathrm{C}$ for $5-10$ minutes and centrifuge at $13,000 \mathrm{xg}$ for 2 minutes at $4^{\circ} \mathrm{C}$. Note: This DNA solution is ready for qPCR assays. Pipette 2-5ul supernatant into a 20ul PCR Master Mixture and run PCR/Real-Time PCR at thermal cyclers.
- Add 100 ul DNA precipitation solution into the tubes, and mix thoroughly by pipette up and down several times. Transfer the suspension into a DNA spin column with 1.5 ml collection tube.
- Centrifuge at $13,000 \mathrm{xg}$ for 10 minutes at $4^{\circ} \mathrm{C}$. Aspirate liquid and simply wash pellet with $80 \%$ ethanol for 2 times (don't resuspend the DNA pellets). Spin down the pellet if resuspended.
- Air-dry pellet for 5-10 minutes and dissolve DNA in 50ul TE buffer or distilled water. Measure the DNA concentration with 260/280nm spectrometer and store DNA solution at- $20^{\circ} \mathrm{C}$.
- Pipette 1-5ul DNA solution into a 20ul PCR Master Mixture and run PCR/Real-Time PCR at thermal cyclers.


## Technical highlights:

- Nuclei isolation is an important step for removing the cytoplasmic proteins, cytosolic DNA and other organelles, able to reduce IP backgrounds and enhance the targeted antibody-protein binding.
- Sonication optimizations: avoid too much foaming formation and overheat the samples by changing either the power settings or/and the number of pulses. Keep samples on ice and wait for 30-60

[^1]seconds between sonication pulses. Run a gel to check the sonicated DNA sizes and visualize the shearing efficiency. The ideal sheared DNA fragments should be around 500-700bp.

- Antibodies-protein binding and qPCR optimizations: do duplicated IP assays using 0.2 ug and 2 ug of antibody to optimize the binding conditions and maximize the yields of specific immuno-complexes. Do duplicated qPCR reactions using 1 ul and 5ul of DNA to quantify DNA signals. Normalize both Ct values of the CHIP DNA sample to the Ct value of the input DNA sample to account for the differences of chromatin sample preparation.
- An innovative, fast DNA purification procedure ( 30 minutes): No toxic chemical involved (e.g. phone-chloroform) and no spin column purification steps are able to avoid the loss of targeted DNA.
- Quantification of cofactors or related proteins on micron-well: add the first/second antibodies (1:1000 dilution) into the micro-wells, prepared from step 5. Incubated at room temperature for 45 minutes and developed by $\mathrm{TMB} / \mathrm{H}_{2} \mathrm{SO}_{4}$ substrates. Measure the protein concentration by O.D 450nm.
- Quantification of CHIP DNA on micron-well: add 10 ul of zmtech Fluo-DNA loading buffer (LB-001) and 90ul of PBS (1x) into the micron-well, prepared from step 5. Incubated at room temperature for 5 minutes with gentle shaking. Wash the micron-wells with 200ul washing buffer for 3 times, and aspirate liquids by taping the micro-wells on absorbent paper towel. (The remained unbound fluorescence were washed out). Add $50-100 \mathrm{ul}$ of $\mathrm{PBS} / \mathrm{TBS}$ and measure the OD . value at 480 nm with plate reader or spectrophotometry.
- Prepare the micron-wells coating with Protein A/G beads: (optional)

Step 1. Add 100 ul of immobilizing buffer and $\underline{2 u l}$ of protein $\mathrm{A} / \mathrm{G}(0.2-2 \mathrm{ug})$ into the micron-wells. Mix thoroughly by pipette up and down several times. Cover the wells with adhesive sealing film or Parafilm. Incubate at $4^{\circ} \mathrm{C}$ for overnight or room temperature for 1 hour.
Step 2. Wash the micro-wells 3 times with 200ul washing buffer. Aspirate liquids by taping the micron-wells on absorbent paper towel.
Step 3. Add 100 ul of IP buffer and $\underline{2 u l}$ of the interested antibody ( $0.2-2 \mathrm{ug}$ ) or normal rabbit IgG (Negative control) or Anti-RNA Polymerase II(Positive control) into the micron-wells. (Recommended Antibody Dilutions: 1:50)
Step 4. Cover the wells with adhesive sealing film and incubate at room temperature for 1 hour with gentle shaking. Aspirate liquids by taping the micron-wells on absorbent paper towel.
Step 5. Wash wells 3 times with 200ul washing buffer. Aspirate liquids by taping the micron-wells on absorbent paper towel.
Step 6. Add 100 ul IP buffer into the wells. Incubate for 30 minutes at room temperature with gentle shaking. The wells are ready to perform IP assays.

- Comparison of the zmtech M/G-fCHIP Kit with other commercial CHIP kits

|  | Zmtech m-fCHIP kit | Commercial/homemade CHIP kits |
| :---: | :--- | :--- |
| 1. Starting material: | 1000-10e6 cells <br> small tissue biopsies/cells <br> embryonic cells | $10 \mathrm{e} 6-10 \mathrm{e}$ cells <br> large amount of cells/tissues |
| 2. Length of protocol: | $2-3$ hours <br> fewer steps and reagents | 7 hours-4 days <br> long steps and large buffers involved |
| 3. DNA isolation: | 30 minutes <br> (Environmental friendly reagents) | $1.5-4$ hours <br> (Phenol/chloroform) |
| 4. Antibody required: | 0.2-2ug <br> (No limit to CHIP-grade antibodies) | 2-10ug <br> (CHIP-grade antibodies) |
| 5. Equipment required: | Routine | Magnetic equipment/ultrasonic water bath |
| 6. Protein A/G beads: | Optional | Required |
| 7. IP to DNA isolation: | Single well procedure | Multiple tubes |
| 8. Quantitative assays: | qPCR, ELISA, western blots | qPCR |
| 9. Applications: | multiple genomic sites analysis <br> multiple related proteins/cofactors analysis |  |

Flow Chart of CHIP assay: (an innovative nuclei isolation/DNA purification technology)

1. Antibodies coating in microwells (overnight $/ 4^{\circ} \mathrm{C}$ )
2. Cross-link and quenching ( 15 mins )
3. Nuclei preparation and Sonication ( 15 mins )
4. Antibodies Immunoprecipitation (IP) ( 1 hrs )
5. Cross-link reversal and DNA purification (30 mins)
6. qPCR quantitation


Wash 3 times with washing buffer (200ul)

## Protein/DNA quantification

1) 1 st/2nd antibodies $\mathrm{TMB} / \mathrm{H}_{2} \mathrm{SO}_{4}$
(Color development)
2) Fluo-DNA (LB-001) (fluorescence DNA assay)


Precautions and Disclaimer: This product and procedure described are intended for R\&D use only. Purchase of this product does not convey a license to perform any patented process.


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