Product Information

M-Fast® Chromatin immunoprecipitation Kit

Catalog Number: m-CHIP-02 (16 Assays) **Stored at 2-8°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) dissolved in 2.0 mL distilled water prior to use	Interested antibodies (0.2-1ug) Positive/negative control antibodies (0.2ug)
Micron-well strip (32 wells) with adhesive sealing films	37% Formaldehyde solution (300 ul)
Nuclei isolation buffer (300ul)	1M Glycine solution (1.2mL)
IP Buffer (10ml) contained protease inhibitors	Primers for PCR/qPCR assays
1x washing buffer (25ml)	PCR/qPCR reagents
DNA isolation reagent-A (1.0 ml), B (100ul), C (200ul)	1xPBS/TBS
Adhesive sealing films (6 sheets)	Gel analysis reagents/qPCR machine

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

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

- Add 100ul of immobilizing buffer and 2ul of the interested antibody (0.2-2ug) or normal rabbit IgG (Negative Control) or Anti-RNA Polymerase II(Positive Control) into the micro-wells. 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°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 200ul washing buffer (1X). Aspirate liquids by taping the micron-wells on absorbent paper towel.
- Add 100ul 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 micron-wells coating with Protein A/G beads).**

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

- Add 15ul of 37% Formaldehyde solution directly into the cell culture tube/dish/microplate well containing 500ul media (approximately 1-10x10e5 cells/5-20mg 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 500ul PBS. Then, add 15ul of 37% formaldehyde and incubate for 15 minutes at room temperature with gentle shaking.

- Add 62ul of ice cold 1M 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 250ul ice-cold washing buffer. Aspirate liquids.
- Add 250ul IP buffer and 15ul 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

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°C. Discard the supernatant.
- Add 200ul 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 xg for 10 minutes at 4°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°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 45-60ul 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°C incubator with gentle shaking. Aspirate liquids by taping the micron-wells on absorbent paper towel.
- Wash wells 3 times with 200ul 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 50ul DNA isolation reagent-A and 5ul 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°C-60°C in the water bath or hybridization oven for 15 minutes. Mix thoroughly by pipette up and down several times every 5 minutes.
- Add 10ul DNA isolation reagent-C into the Micron-wells and mix thoroughly by pipette up and down several times. Transfer the suspension into a clean 0.5ml/1.5ml tube.
- Incubate at 95°C for 5-10 minutes and centrifuge at 13,000 xg for 2 minutes at 4°C.

Note: This DNA solution is ready for PCR/qPCR assays or continue the DNA precipitation procedure (See technical highlights).

- Transfer the supernatant into a clean 0.2ml/1.5ml tube and stored at -20°C for downstream applications.
- 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 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 0.2ug and 2ug of antibody to optimize the binding conditions and maximize the yields of specific immuno-complexes. Do duplicated qPCR reactions using 1ul 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 TMB/H_2SO_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 50-100ul of PBS/TBS and measure the OD. value at 480nm with plate reader or spectrophotometry.
- **DNA precipitation procedure: (optional)**
  1) Add 100ul Zmtech DNA precipitation solution (cat. PS-01D) into the DNA tubes from step 6., and mix thoroughly by pipette up and down several times. Transfer the suspension into a DNA spin column with 1.5ml collection tube (Cat: SC-01).

  2) Centrifuge at 13,000 xg for 10 minutes at 4°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.

  3) 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°C.
- Prepare **the micron-wells coating with Protein A/G beads:** (optional)

  Step 1. Add 100ul of immobilizing buffer and 2ul of protein A/G (0.2-2ug) 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°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 100ul of IP buffer and 2ul of the interested antibody (0.2-2ug) 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.

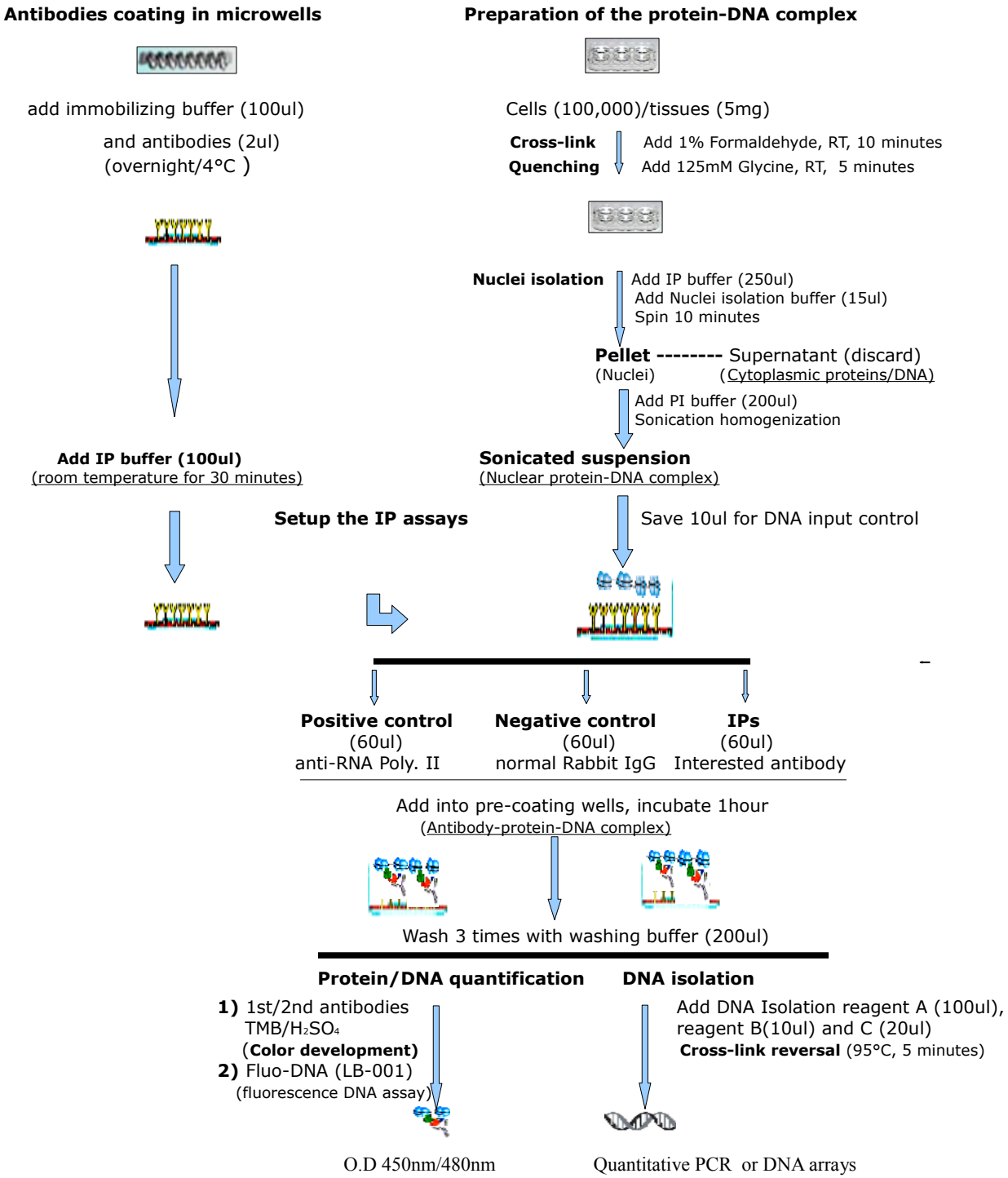
  Step 5. Wash wells 3 times with 200ul washing buffer. Aspirate liquids by taping the micron-wells on absorbent paper towel.

  Step 6. Add 100ul 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 small tissue biopsies/embryonic cells	10e6-10e7 cells large amount of cells/tissues
2. Length of protocol:	2-3 hours fewer steps and reagents	7 hours- 4 days long steps and large buffers involved
3. DNA isolation:	30 minutes (Environmental friendly reagents)	1.5-4 hours (Phenol/chloroform)
4. Antibody required:	0.2-2ug (No limit to CHIP-grade antibodies)	2-10ug (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/spin column transfers
8. Quantitative assays:	PCR/qPCR, ELISA, western blots	PCR/qPCR
9. Applications:	multiple genomic sites analysis; multiple related proteins/cofactors analysis.	

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

1. **Antibodies coating in microwells** (overnight/4°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. **PCR/qPCR quantitation**



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