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EpiQuik™ Chromatin Immunoprecipitation Kit

Base Catalog # P-2002

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ Chromatin Immunoprecipitation Kit is suitable for combining the specificity of immunoprecipitation with qualitative and quantitative PCR, MS-PCR, DNA sequencing, and southern blot, as well as DNA microarray.

Like using other ChIP kits, if you use the EpiQuik™ ChIP kits, choice of good antibody (i.e. IP proven) is required for precipitating the fixed protein/DNA complexes.

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KIT CONTENTS

Important Information: The amount of components supplied in this kit is designed for reaction count, not sample count, such as negative IgG controls and input DNA. Thus, experiments with samples to be paired with both IgG and input may require additional columns or components to be purchased separately. Please calculate the necessary volumes based on the below kit contents and protocol prior to starting the experiment.

Components	24 reactions P-2002-1	48 reactions P-2002-2	96 reactions P-2002-3
CP1 (Wash Buffer)	28 ml	2 x 28 ml	4 x 28 ml
CP2 (Antibody Buffer)	15 ml	30 ml	2 x 30 ml
CP3A (Pre-Lysis Buffer)	4 ml	8 ml	16 ml
CP3B (Lysis Buffer)	4 ml	6 ml	10 ml
CP4 (ChIP Dilution Buffer)	4 ml	6 ml	10 ml
CP5 (DNA Release Buffer)	2 ml	2 x 2 ml	6 ml
CP6 (Reverse Buffer)	2 ml	2 x 2 ml	6 ml
CP7 (Binding Buffer)	5 ml	8 ml	15 ml
CP8 (Elution Buffer)	0.6 ml	1.2 ml	2 ml
Protease Inhibitor Cocktail (100X)*	25 µl	40 µl	80 µl
Non-immune IgG (1 mg/ml)*	10 µl	10 µl	20 µl
Anti-RNA Polymerase II (1 mg/ml)*	5 µl	5 µl	10 µl
Proteinase K (10 mg/ml)*	25 µl	50 µl	100 µl
Control Primer (GAPDH)			
Forward (20 µM)*	10 µl	15 µl	20 µl
Reverse (20 µM)*	10 µl	15 µl	20 µl
8-Well Assay Strips (with Frame)	3	6	12
8-Well Strip Caps	3	6	12
F-Spin Column	30	50	100
F-Collection Tube	30	50	100
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** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store the following components at 4°C: **Protease Inhibitor Cocktail, Non-immune IgG, Anti-RNA Polymerase II, Proteinase K, Control Primers** and **8-Well Assay Strips.** (2) Store **all other components** at room temperature.

The kit is stable for up to 6 months from the shipment date, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Variable temperature waterbath
- ☐ Vortex mixer
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Sonicator
- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 15 ml conical tube
- ☐ Antibodies of interest
- ☐ 37% formaldehyde
- ☐ Glycine solution
- ☐ TE buffer (pH 8.0)
- ☐ Ethanol (96-100%)

GENERAL PRODUCT INFORMATION

Quality Control: Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design.

Usage Limitation: The *EpiQuik*™ ChIP kits are for research use only and are not intended for diagnostic or therapeutic application.

Intellectual Property: The *EpiQuik*™ ChIP kits and methods of use contain proprietary technologies by Epigentek. *EpiQuik*™ is a trademark of Epigentek Group Inc.

A BRIEF OVERVIEW

Protein-DNA interaction play a critical role for cellular functions such as signal transduction, gene transcription, chromosome segregation, DNA replication and recombination, and epigenetic silencing. Identifying the genetic targets of DNA binding proteins and knowing the mechanisms of protein-DNA interaction is important for understanding cellular process.

Chromatin Immunoprecipitation (ChIP) offers an advantageous tool for studying protein-DNA interactions. Unlike other methods such as EMASA, DNA microarrays, and report gene assays- which analyze direct interactions between protein and DNA *in vitro*- ChIP can detect that a specific protein binds to the specific sequences of a gene in living cells. There are several methods used for

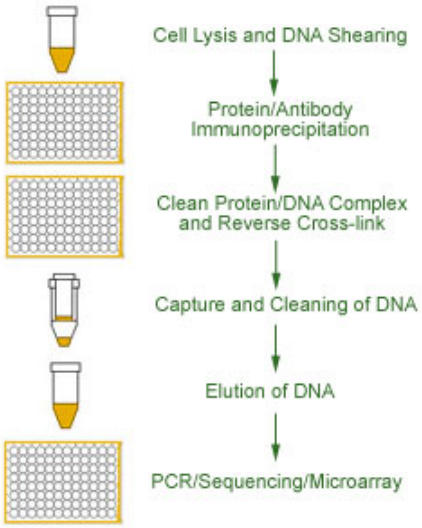
chromatin immunoprecipitation. However, most of these methods available so far are considerably time consuming, labor intensive, or have low throughput.

The *EpiQuik*™ Chromatin Immunoprecipitation kits use a proprietary and unique procedure/composition to investigate protein-DNA interaction *in vivo* efficiently. The *EpiQuik*™ Chromatin Immunoprecipitation Kit series have the following features:

- The fastest procedure available, which can be finished within 5 hours.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Columns for DNA purification are included, which save time and reduce labor.
- Compatible with all DNA amplification-based approaches.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ Chromatin Immunoprecipitation Kit contains all reagents required for carrying out a successful chromatin immunoprecipitation from mammalian cells. Particularly, this kit includes a positive control antibody (RNA polymerase II), a negative control Non-immune IgG, and GAPDH primers that can be used as a positive control to demonstrate the efficacy of the kit reagents and protocol. RNA polymerase II is considered to be enriched in the GAPDH gene promoter that is expected to be undergoing transcription in most growing mammalian cells, and can be immunoprecipitated by RNA polymerase II, but not by Non-immune IgG. In this ChIP, cells are cross-linked with formaldehyde and chromatin is extracted. The chromatin is then sheared and added into the microwell and immobilized with affinity antibodies. Cross-linked DNA is released from the antibody-captured protein-DNA complex, reversed, and purified through the specifically designed Fast-Spin Column. Eluted DNA can be used for various down-stream applications.



Schematic Procedure for Using the *EpiQuik*™ Chromatin Immunoprecipitation Kit

PROTOCOL

Before starting, perform the following:

1. Prepare the following required solutions (not included): 90% Ethanol; 70% Ethanol; 1.25 M Glycine Solution; and 1X TE Buffer (pH 8.0).
2. Ensure that all buffers are in clear solution. Shake or vortex if these buffers precipitate.

Note: When processing spin columns, always cap spin columns before placing them in the microcentrifuge.

Antibody Binding to the Assay Plate

1. Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Wash strip wells once with 150 µl of **CP1**.
2. Add 100 µl of **CP2** to each well and then add the antibodies: 1 µl of **Non-immune IgG** as the negative control, 1 µl of **Anti-RNA Polymerase II** as the positive control, and 2-4 µg of each *antibody of interest*.
3. Cover the strip wells with Parafilm M and incubate at room temperature for 60-90 minutes. Meanwhile, prepare the cell extracts as described in the next steps.

Cell Collection and *In Vivo* Cross-Link

For Monolayer or Adherent Cells:

1. Cells (treated or untreated) are grown to 80%-90% confluency on a 100 mm plate, about 2-4 × 10^6 cells (0.5 × 10^6 cells are required for each reaction), then trypsinized and collected into a 15 ml conical tube. Count cells in a hemacytometer.
2. Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Wash cells with 10 ml of PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.
3. Add 9 ml fresh culture medium containing *1% formaldehyde* (final concentration) to cells. Incubate at room temperature (20-25°C) for 10 minutes on a rocking platform (50-100 rpm).

For Suspension Cells:

1. Collect cells (treated or untreated) into a 15 ml conical tube. (1-2 × 10^6 cells are required for each reaction). Count cells in a hemacytometer.
2. Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Wash cells with 10 ml of PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.
3. Add 9 ml fresh culture medium containing *1% Formaldehyde* (final concentration) to cells. Incubate at room temperature (20-25°C) for 10 minutes on a rocking platform (50-100 rpm).

Cell Lysis and DNA Shearing

1. Add 1 ml of *1.25 M Glycine solution;* mix and centrifuge at 1000 rpm for 5 minutes. Remove medium and wash cells once with 10 ml of ice cold PBS by centrifuging at 1000 rpm for 5 minutes.

2. Add **CP3A** to re-suspend the cell pellet (200 µl/1 × 10^6 cells for adherent cells and 100 µl/1 × 10^6 cells for suspension cells). Transfer cell suspension to a 1.5 ml vial and incubate on ice for 10 minutes. Vortex vigorously for 10 seconds and centrifuge at 5000 rpm for 5 minutes.
3. Carefully remove supernatant. Add **CP3B** containing **Protease Inhibitor Cocktail (PIC)** (Ex: 10 µl of **PIC** to each 1 ml of **CP3B**) to re-suspend the nuclear pellet (50-100 µl/1 × 10^6 cells, 500 µl maximum for each vial). Incubate the sample on ice for 10 minutes and vortex occasionally.
4. Shear DNA by sonication. Usually, sonicate 3 to 4 pulses of 10 to 12 seconds each at level 2 using a Branson Microtip probe, followed by 30 to 40 seconds rest on ice between each pulse. (*The conditions of crosslinked DNA shearing can be optimized based on cells and sonicator equipment. If desired, remove 5 µl of sonicated cell lysate for agarose gel analysis. The length of sheared DNA should be between 200-1000 bp.*)
5. Pellet cell debris by centrifuging at 14,000 rpm for 10 minutes.

Protein/DNA Immunoprecipitation

1. Transfer clear supernatant to a new 1.5 ml vial (supernatant can be stored at –80°C at this step). Dilute required volume of supernatant with **CP4** at a 1:1 ratio (ex: add 100 µl of **CP4** to 100 µl of supernatant).
2. Remove 5 µl of the *diluted supernatant* to a 0.5 ml vial. Label the vial as "input DNA", and then place on ice.
3. Remove the incubated antibody solution and wash the strip wells three times with 150 µl of **CP2** by pipetting in and out.
4. Transfer 100 µl of the *diluted supernatant* to each strip well. Cover the strip wells with Parafilm M and incubate at room temperature (22-25°C) for 60-90 minutes on an orbital shaker (50-100 rpm).
5. Remove supernatant. Wash the wells six times with 150 µl of **CP1**. Allow 2 minutes on an orbital shaker (100 rpm) for each wash. Wash the wells once (for 2 minutes) with 150 µl of *1X TE Buffer*.

Cross-Linked DNA Reversal/DNA Purification

1. Add 1 µl of **Proteinase K** to each 40 µl of **CP5** and mix. Add 40 µl of **CP5** containing **Proteinase K** to the samples (including the "input DNA" vial). Cover the sample wells with strip caps and incubate at 65°C in a waterbath for 15 minutes.
2. Add 40 µl of **CP6** to the samples, mix, and re-cover the wells with strip caps and incubate at 65°C in a waterbath for 90 minutes. Also add 40 µl of **CP6** to the vial containing *supernatant* labeled as "input DNA." Mix and incubate at 65°C for 90 minutes.
3. Place a spin column into a 2 ml collection tube. Add 150 µl of **CP7** to the samples and transfer mixed solution to the column. Centrifuge at 12,000 rpm for 20 seconds.
4. Add 200 µl of *70% ethanol* to the column, centrifuge at 12,000 rpm for 15 seconds. Remove the column from the collection tube and discard the flowthrough.
5. Replace column to the collection tube. Add 200 µl of *90% ethanol* to the column and centrifuge at 12,000 rpm for 20 seconds.
6. Remove the column and discard the flowthrough. Replace column to the collection tube and wash the column again with 200 µl of *90% ethanol* at 12,000 rpm for 35 seconds.
7. Place the column in a new 1.5 ml vial. Add 10-20 µl of **CP8** directly to the filter in the column and centrifuge at 12,000 rpm for 20 seconds to elute purified DNA.

DNA is now ready for use or storage at –20°C.

Note: *If a conventional PCR or a SYBR green real time PCR is performed, control primers (110 bp, for human cells) included in the kit can be used as positive control. For conventional PCR, the number of PCR cycles may need to be optimized for better PCR results.*

In general, the amplification difference between "normal IgG control" and "positive control" may vary from 3 to 8 cycles, depending on experimental conditions.

TROUBLESHOOTING

Little or No PCR Products

1. Insufficient cell number.	Increase cell number (ex: >0.5 million).
2. Insufficient or too much cross-linking.	Check if the appropriate cross-link step is carried out according to the protocol.
3. Insufficient cell lysis.	Follow the guidelines in the protocol. Check the cell lysis by observing a 5 µl portion of the cell lysate under the microscope for intact cells.
4. Insufficient/too much sonication.	Follow the protocol instruction for obtaining the appropriate sized DNA. Keep the sample on ice during the sonication.
5. Antibody doesn't bind to protein.	Check if the subclass or isotype of the antibody is correct. Choose an antibody that is ChIP or IP grade.
6. Incorrect temperature/insufficient time for DNA release and reversal of cross-linking.	Follow the guidelines in the protocol for appropriate temperature and time.
7. Incorrect PCR conditions.	Check if all PCR components are added. Increase amount of DNA added to PCR reaction. Increase the number of cycles for PCR reaction.
8. Wrong or bad primers.	Ensure the designed primers are specific to the target sequence.
9. The column is not washed with 90% ethanol.	Ensure that wash solution is 90% Ethanol.

10. DNA is not completely passed through the filter.	Increase centrifuge time to 1 minute at steps 3 to 7 of "Cross-Linked DNA Reversal/DNA Purification."

Little or No Amplification Difference Between the Positive Control and the Negative Control

1. Insufficient wash at each wash step.	Check if washing recommendations at each step is performed according to the protocol.
2. Positive control antibody is added into the well for the negative control by mistake.	Ensure the antibodies are added into the correct wells.
3. Too many PCR cycles.	If using conventional PCR, decrease the cycles to appropriate cycle number. Differences between quantities of starting DNA can be measured generally within the linear PCR amplification phase.

RELATED PRODUCTS

P-2003	*EpiQuik*™ Tissue Chromatin Immunoprecipitation (ChIP) Kit
P-2006	*EpiQuik*™ Methyl-Histone H3-K9 ChIP Kit
P-2007	*EpiQuik*™ Methyl-Histone H3-K4 ChIP Kit
P-2008	*EpiQuik*™ Tissue Methyl-Histone H3-K9 ChIP Kit
P-2009	*EpiQuik*™ Tissue Methyl-Histone H3-K4 ChIP Kit
P-2010	*EpiQuik*™ Acetyl-Histone H3 ChIP Kit
P-2011	*EpiQuik*™ Acetyl-Histone H4 ChIP Kit
P-2015	*EpiQuik*™ Methyl-Histone H3-K27 ChIP Kit
P-2016	*EpiQuik*™ Tissue Methyl-Histone H3-K27 ChIP Kit
P-2017	*EpiQuik*™ Methyl-CpG Binding Domain Protein 2 ChIP Kit
P-2018	*EpiQuik*™ Tissue Methyl-CpG Binding Domain Protein 2 ChIP Kit

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