Store at -20°C

SimpleChIP® Universal qPCR Master Mix

#88989

1 mL (1 x 1 mL)
5 mL (5 x 1 mL)

Cell Signaling TECHNOLOGY®

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

For Research Use Only. Not For Use In Diagnostic Procedures.

Background: Dye-based quantitative PCR (qPCR) uses real-time fluorescence of a double-stranded DNA (dsDNA) binding dye, most commonly SYBR® Green I, to measure DNA amplification as it occurs during each cycle of PCR. At a point where the fluorescence signal is confidently detected over the background fluorescence, a quantification cycle, or C_T value, can be determined. C_T values can be used to evaluate relative target abundance between two or more samples, or to calculate absolute target quantities in reference to an appropriate standard curve, derived from a series of known dilutions. qPCR is commonly used to detect and quantify target genes in genomic DNA that is enriched by chromatin immunoprecipitation (ChIP) or Cleavage Under Targets and Release Using Nuclease (CUT&RUN).

Description: The SimpleChIP® Universal qPCR Master Mix is an optimized 2X reaction mix for real-time qPCR detection and quantitation of target DNA sequences using the SYBR/FAM channel of most real-time qPCR instruments. It contains Hot Start Taq DNA Polymerase and has been formulated with a unique passive reference dye that is compatible across a variety of instrument platforms (including those that require a high or low ROX reference signal). It also features dUTP for carryover prevention and a non-fluorescent, visible dye to monitor reaction setup. This dye does not spectrally overlap with fluorescent dyes used for qPCR and will not interfere with real-time detection.

This product is provided in 1 ml volumes sufficient for preparation of 100 qPCR reactions, and is compatible with both enzymatic and sonication-fragmented DNA samples from SimpleChIP® enzymatic and sonication ChIP kits and CUT&RUN Assay Kit #86652. This master mix formulation is supplied at 2X concentration and contains all PCR components required for amplification and quantitation of DNA, except primers and a DNA template.

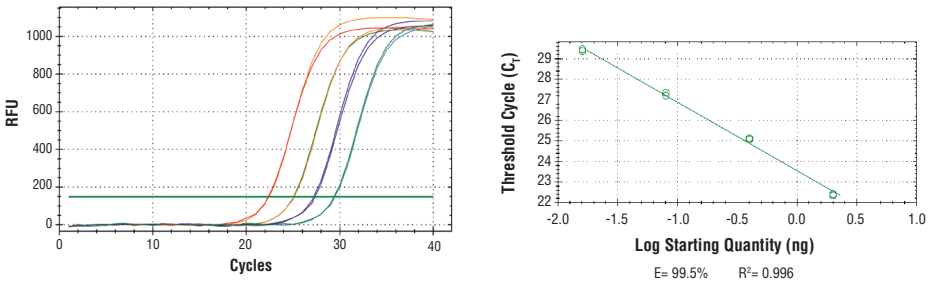
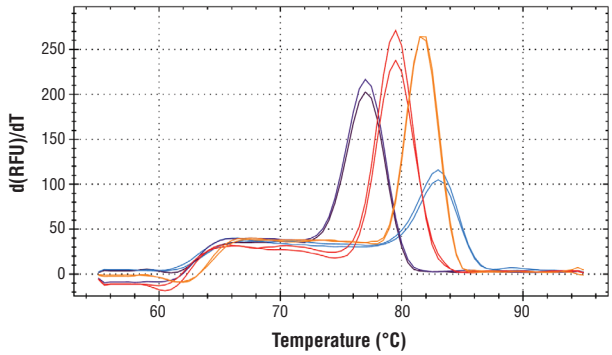


Figure 1. *Amplification plot (left) and regression curve (right) of a serial dilution of input DNA samples. Input DNA was prepared and purified from HCT 116 cells using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. Quantitative PCR was performed with 20 (red line), 4 (orange line), 0.8 (purple line), and 0.016 (blue line) ng of input DNA and SimpleChIP® Human RPL30 Exon 3 Primers #7014. Data is shown for two replicates.*



◀ ***Figure 2.*** *Corresponding single melt peak of PCR products. Quantitative PCR was performed with input DNA sample from HCT 116 cells prepared by SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005 and either SimpleChIP® Human α Satellite Repeat Primers #4486 (red line), SimpleChIP® Human RPL30 Exon 3 Primers #7014 (orange line), SimpleChIP® Human CaMK2D Intron 3 Primers #5111 (purple line), or human c-MYC promoter primers (blue line). Data for two replicates is shown for each primer set.*

Storage: This product is stable for 12 months when stored at -20°C and protected from light. It is stable for up to 30 freeze/thaw cycles and can be stored at 4°C, protected from light, for up to 1 month.

Compatible Assay kits:

SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) #9002

SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003

SimpleChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads) #9004

SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005

SimpleChIP® Plus Sonication Chromatin IP Kit #56383

CUT&RUN Assay Kit #86652

Required Reagents:

Reagents Included:

SimpleChIP® Universal qPCR Master Mix (2X) #88989

Reagents Not Included:

a. Nuclease-free Water #12931
b. Target-specific primers
c. qPCR instrument
d. qPCR plates and seals
e. PCR strip tubes or microcentrifuge tubes

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

NEW ENGLAND BioLabs Inc. Contains: NEB® Luna® qPCR reagent

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

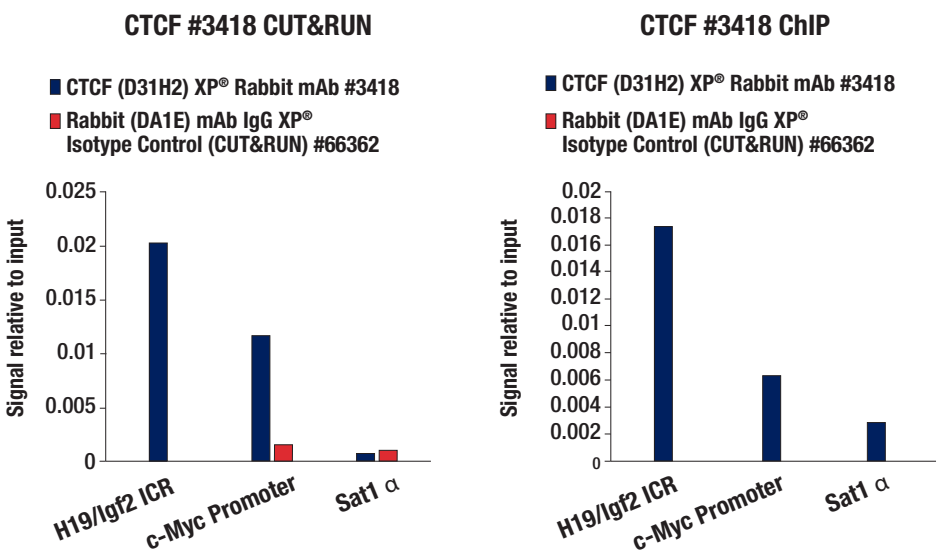

Figure 3. *CUT&RUN and ChIP assays were performed with HCT 116 cells and CTCF (D31H2) XP® Rabbit mAb #3418 or Rabbit (DA1E) mAb IgG XP® Isotype Control (CUT&RUN) #66362, using CUT&RUN Assay Kit #86652 (left panel) or the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005 (right panel). The enriched DNA was quantified by real-time PCR using this SimpleChIP® Universal qPCR Master Mix, human c-Myc promoter primers, SimpleChIP® Human H19/Igf2 ICR Primers #5172, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.*

Quantification of DNA by real-time quantitative PCR

Recommendations

- The "SYBR® Green" or "SYBR®/FAM" channel of the real-time instrument should be used for the SimpleChIP® Universal qPCR Master Mix. On some instruments, selecting a single channel for data collection can result in faster experiment times.
- Ensure that all components are thawed and mixed prior to use. Once thawed, place on ice. For best results, reactions should be kept on ice prior to thermo cycling.
- Run reactions in duplicate or triplicate for each sample. This permits exclusion of outlier traces due to unexpected plate issues, edge effects, or other problems, while maintaining accurate quantitation.
- qPCR is a sensitive DNA detection method. Use filtered pipette tips to minimize risk of contamination.
- When using multichannel pipettes, care should be taken to ensure consistency of volume dispensed.
- When pipetting into the qPCR plate, it is advisable to avoid the formation of bubbles. If 1–2 small bubbles are present at the top of the liquid after loading, the assay can proceed, as these bubbles will typically resolve during the first denaturation step of the PCR. Excess bubbles can be removed by brief centrifugation.
- A denaturation or melt curve step should be added at the end of the qPCR cycling protocol to evaluate amplification specificity.
- Primers purified with standard desalting are sufficient for use in SimpleChIP® qPCR. HPLC or PAGE purification may be helpful for assays that require increased sensitivity.
- PCR primer selection is critical. The use of qPCR primer design software (e.g., Primer3) maximizes the likelihood of amplification success while minimizing nonspecific amplification and primer dimers. Targets with balanced GC/AT content (40–60%) tend to amplify efficiently. Where possible, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases (to avoid potential off-target amplification). Primers should be designed with close adherence to the following criteria:

Primer length:	*24 nucleotides*
Optimum Tm:	*60°C*
Optimum GC:	*50%*
Amplicon size:	*80 to 160 bp*

- For most targets, a final concentration of 500 nM (each primer) will provide optimum performance. If needed, primer concentrations can be optimized between 100–500 nM.

Before Starting

- Thaw primer sets, 2% input DNA, ChIP-DNA, or CUT&RUN DNA, and SimpleChIP® Universal qPCR Master Mix (2X) #88989, then place on ice. After thawing, completely mix each component by inversion, pipetting, or gentle vortexing.
- Make serial dilutions of input DNA (undiluted, 1:5, 1:25, 1:125) that will be used to create a standard curve to determine the efficiency of amplification and quantify relative enrichment of target loci. These can be diluted in either water or TE.

1. Label the appropriate number of PCR tubes or PCR plates compatible with the model of PCR machine to be used. In addition to ChIP DNA or CUT&RUN DNA samples, PCR reactions should also include a no template DNA control (NTC) for contamination control, and a serial dilution of the input chromatin DNA (undiluted, 1:5, 1:25, 1:125).

2. Add 2 µl of the appropriate DNA sample to each tube or well of the PCR plate.

3. Prepare a master reaction mix as described below. Set up 2-3 PCR replicates for each DNA sample. Volumes shown are for a 20 µl reaction, which is recommended for 96-well plates and tubes. For 384-well plates, a final reaction volume of 10 µl is recommended. Add enough reagents to account for loss of volume. Add 18 µl of reaction mix to each PCR reaction tube or well.

Reagent	Volume for 1 PCR Reaction (18 µl)
Nuclease-free water	*6 µl*
Primers (5 µM)	*2 µl*
SimpleChIP® Universal qPCR Master Mix (2X) #88989	*10 µl*

4. Mix PCR reactions thoroughly but gently by pipetting or vortexing.

5. Seal tubes with flat, optically transparent caps. Seal plates with optically transparent film. Care should be taken to properly seal plate edges and corners to prevent artifacts caused by evaporation.

6. Collect liquid to the bottom of the tube or plate by brief centrifugation.

7. Start the following PCR reaction program. Ensure a plate read is included at the end of the extension step, and a denaturation (melt) curve after cycling is complete to analyze product specificity. Use the SYBR® or SYBR®/FAM scan mode setting on the real-time instrument. Optimal anneal temperature may vary for different primer sets and may need to be determined empirically.

a.	*Initial Denaturation*	*95°C 3 min*
b.	*Denature*	*95°C 15 sec*
c.	*Anneal and Extension*	*60°C 60 sec*
d.	*Repeat steps b and c for a total of 40 cycles.*	
e.	*Melt Curve step from 60-95°C.*	

8. Determine the efficiency of the standard curve by plotting the log of the input concentration against the C_T. This can be done automatically using most qPCR instrument software. The linear fit of this data should have a slope of -3.6 to -3.1, corresponding to an acceptable PCR efficiency of 90-110% and a correlation coefficient (R^2) value of ≥ 0.98.

9. Determine the PCR amplification specificity by evaluating the melt curve profile. Specific amplification of the target sequence will generate a single melt peak. The melt profiles of the NTC samples should also be evaluated. The presence of NTC profiles that overlap the positive sample profiles may indicate the presence of contamination. Any samples whose melt profiles overlap the profile of NTC samples should not be evaluated.

10. Quantify experimental samples relative to an input control or standard curve using the software provided with the real-time PCR machine. Alternatively, one can calculate the IP efficiency manually using the Percent Input Method and the equation shown below. With this method, signals obtained from each immunoprecipitation are expressed as a percent of the total input chromatin.

Percent Input = 2% x $2^{(C[T]\ 2\%Input\ Sample\ -\ C[T]\ IP\ Sample)}$

C[T] = C_T = Average threshold cycle of PCR reaction

APPENDIX: Toubleshooting Guide

Problem	Possible Causes	Recommendation
1. qPCR traces show low or no amplification.	*Poor ChIP or CUT&RUN DNA sample or primer design.*	*ChIP or CUT&RUN DNA should be purified using a DNA purification column or equivalent method. Unpurified DNA gives very poor amplification.*
		Low or no amplification of purified input DNA is indicative of a poor primer set and optimization or redesign of the primer set may be needed.
		Efficient amplification of input DNA and absence of amplification of ChIP or CUT&RUN sample DNA is indicative of a failed or ChIP or CUT&RUN assay.
	Reagent added improperly or omitted from qPCR assay.	*Verify all steps of the protocol were followed correctly.*
	Incorrect cycling protocol.	*Refer to the proper qPCR cycling profile in this protocol.*
	Incorrect reporter dye selected for the qPCR thermal cycler.	*Select FAM/SYBR® channel on the qPCR instrument.*
	Reagents are contaminated or degraded.	*Confirm the expiration dates of the kit reagents and verify proper storage conditions. Rerun the qPCR assay with fresh reagents.*
2. Inconsistent qPCR traces for triplicate data.	*Improper pipetting during qPCR assay set-up.*	*Ensure proper pipetting techniques.*
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates.	*Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler. Exclude problematic trace(s) from data analysis.*
	Poor mixing of reagents during qPCR set-up.	*Make sure all reagents are properly mixed after thawing them.*
	Bubbles cause an abnormal qPCR trace.	*Avoid bubbles in the qPCR plate. Centrifuge the qPCR plate prior to running it in the thermal cycler. Exclude problematic trace(s) from data analysis.*

Problem	Possible Causes	Recommendation
3. DNA standard curve has a poor correlation coefficient/ efficiency of the DNA standard curve falls outside the 90–110% range	Presence of outlying qPCR traces.	Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems.
	Improper pipetting during qPCR assay set-up.	Ensure that proper pipetting techniques are used.
	Reaction conditions are incorrect.	Verify that all steps of the protocol were followed correctly.
	Bubbles cause an abnormal qPCR trace.	Avoid bubbles in the qPCR plate. Centrifuge the qPCR plate prior to running it in the thermal cycler.
	Poor mixing of reagents.	After thawing, make sure all reagents are properly mixed.
	Threshold is improperly set for the qPCR traces.	Ensure the threshold is set in the exponential region of qPCR traces. Refer to the real-time instrument user manual to manually set an appropriate threshold.
4. Melt curve shows multiple peaks or different peaks for low input samples.	Non-template amplification is occurring.	Compare melt curve of NTC to samples. Similar melt curves for DNA samples and NTC are indicative of non-template amplification.
	Non-specific amplification is occurring, or infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks.	Redesign primers with a Tm of 60°C or determine the optimal annealing temperature of the primers.
		Perform a primer matrix analysis to determine optimal primer concentrations.
5. No template control qPCR trace shows amplification, NTC C_T is close to or overlapping lowest input DNA titration standards.	Reagents are contaminated with DNA (Melt curve of NTC matches melt curve of Input DNA standards).	Replace all stocks and reagents. Clean equipment and setup area with a 10% chlorine bleach.
	Primers produce nonspecific amplification (Melt curve of NTC does not match melt curve of Input DNA standards).	Redesign primers with a Tm of 60°C or use qPCR primer design software.