

SimpleChIP® Human RUNX2 Promoter Primers

✓ 500 µl
(250 PCR reactions)



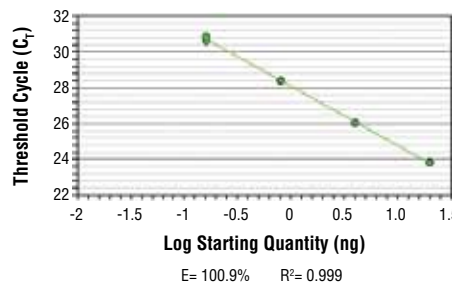
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New 03/13

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

Applications	Species Cross-Reactivity	Primer Anneal/Extension	PCR Product Length
ChIP	H	65°C	114 bp

Description: SimpleChIP® Human RUNX2 Promoter Primers contain a mix of forward and reverse PCR primers that are specific to a region of the human runt-related transcription factor 2 promoter. These primers can be used to amplify DNA that has been isolated using chromatin immunoprecipitation (ChIP). Primers have been optimized for use in SYBR® Green quantitative real-time PCR and have been tested in conjunction with SimpleChIP® Enzymatic Chromatin IP Kits #9002 and #9003 and ChIP-validated antibodies from Cell Signaling Technology®. The RUNX2 gene encodes a protein with a runt-DNA binding domain and has been shown to be a critical component of skeletal morphogenesis and osteoblast differentiation. It has also been shown to regulate its own promoter.



SimpleChIP® Human RUNX2 Promoter Primers were tested on DNA isolated from cross-linked cells using the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. Real-time PCR was performed in duplicate on a serial dilution of 2% total input DNA (20 ng, 4 ng, 0.8 ng, and 0.16 ng) using a real-time PCR detection system and SYBR® Green reaction mix. The PCR amplification efficiency (E) and correlation coefficient (R²) were calculated based on the corresponding threshold cycle (C_t) of each dilution sample during 40 cycles of real-time PCR (95°C denaturation for 15 sec, 65°C anneal/extension for 60 sec).

Storage: Supplied in nuclease-free water at a concentration of 5 µM (each primer is at a final concentration of 5 µM). Store at -20°C.

Directions for Use:

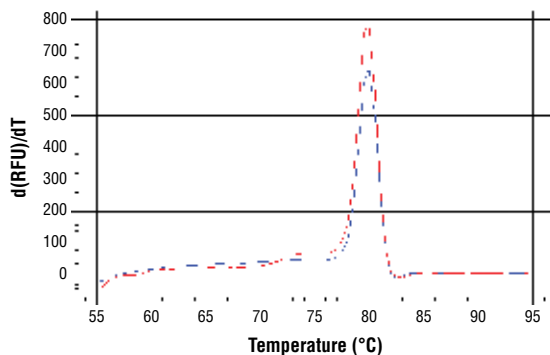
1. Label the appropriate number of PCR tubes or PCR plates compatible with the model of real-time PCR machine to be used. PCR reactions should be performed in duplicate and should include a tube with no DNA to control for contamination, and a serial dilution of a 2% total input chromatin DNA (undiluted, 1:5, 1:25, 1:125), which is used to create a standard curve and determine amplification efficiency.

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

3. Prepare a master PCR reaction mix as described below. Add enough reagents for two extra reactions to account for loss of volume. Add 18 µl of the master PCR reaction mix to each PCR reaction tube or well of the PCR plate.

Reagent	Volume for 1 PCR Reaction (20 µl)
Nuclease-free H ₂ O	6 µl
5 µM SimpleChIP® Primers	2 µl
2X SYBR® Green Reaction Mix	10 µl

4. Start the following PCR reaction program:
- Initial Denaturation: 95°C for 3 min.
 - Denaturation: 95°C for 15 sec.
 - Anneal and Extension: Primer-specific temp. for 60 sec.
 - Repeat steps b and c for a total of 40 cycles.
5. Analyze quantitative PCR results using software provided with the real-time PCR machine.



PCR product melting curves were obtained for real-time PCR reactions performed using SimpleChIP® Human RUNX2 Promoter Primers. Data is shown for duplicate PCR reactions using 20 ng of total DNA. The melt curve consists of 80 melt cycles, starting at 55°C with increments of 0.5°C per cycle. Each peak is formed from the degradation of a single PCR product.

SYBR® Green is a registered trademark of Molecular Probes, Inc.



I. Identification:

Product name: SimpleChIP® Primers

Product Catalog: 4471, 4478, 4486, 4490, 4493, 4641, 4649, 4653, 4659, 4663, 4669, 4779, 4829, 5037, 5047, 5077, 5098, 5111, 5131, 5139, 5148, 5156, 5172, 7014, 7015

CAS#: None

Manufacturer Supplier: Cell Signaling Technology

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Danvers, MA 01923 USA

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978-578-6737 EMERGENCY TEL

II. Composition/Information:

This preparation is composed of deoxyribonucleic acid oligonucleotides in water. Considered non-hazardous.

CAS#: N/A

III. Hazard Identification:

CAUTION: This product is not for use in humans. It is intended for research purposes only. To the best of our knowledge, the chemical, physical, and toxicological properties of this material have not been established.

EMERGENCY OVERVIEW OF PRODUCT

OSHA: No known hazards.

This substance is not classified as dangerous according to Directive 67/548/EEC.

IV. First Aid Measures:

Inhalation: If inhaled, remove to fresh air. If breathing is difficult, get medical attention.

Ingestion: If swallowed, wash out mouth with water provided person is conscious. Get medical attention.

Skin exposure: In case of contact, immediately wash skin with soap and water for at least 15 minutes. Remove contaminated clothing. Wash clothing before reuse.

Eye exposure: In case of contact with eyes, immediately flush eyes with water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Data not available.

Autoignition Temperature: Data not available.

Explosion: Data not available.

Fire extinguishing media: Water spray, dry chemical, alcohol foam, or carbon dioxide.

Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes. May emit toxic fumes under fire conditions.

VI. Accidental Release Measures: Wear appropriate personal protective equipment. Wash spill site thoroughly.

VII. Handling And Storage:

Store in tightly closed container at -20°C. Avoid inhalation. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling.

VIII. Exposure Controls/Personal:

Ventilation System: A system of local and/or general exhaust is recommended.

Skin Protection: Wear compatible chemical resistant gloves and protective clothing.

Eye protection: Wear protective safety glasses or chemical safety goggles. Maintain eye wash fountain and quick-drench facilities in work area.

IX. Physical And Chemical Properties

Appearance:	colorless liquid
Odor:	odorless
pH:	data not available
Melting Point:	data not available
Boiling Point:	data not available
Freezing Point:	data not available
Volatile Organic Compounds:	data not available
Solubility in water:	soluble in water

X. Stability and Reactivity:

Stability: Stable under normal conditions.

Conditions/materials to avoid: Data not available.

Hazardous Decomposition: Data not available.

XI. Toxicological Information:

Acute Effects: Not established.

Chronic Effects: Not established.

Potential Health Effects: Not established.

Inhalation: May be harmful if inhaled.

Skin: May be harmful if absorbed through skin.

Eyes: Causes eye irritation.

Ingestion: May be harmful if swallowed.

XII. Ecological Information: No data available.

XIII. Disposal Considerations: Dispose of in accordance with federal, state, local environmental regulations.

XIV. Transport Information:

DOT: Not dangerous goods.

ADR/RID: Not dangerous goods.

IMDG: Not dangerous goods.

IATA: Not dangerous goods.

XV. Regulatory Information:

EU Regulations/Classifications/Labeling Information: None.

US Regulatory Information:

SARA Listed: No.

Canada (WHMIS): DSL No, NDSL No.

XVI. Other Information:

This compound is sold only for research use only. It is not for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.