



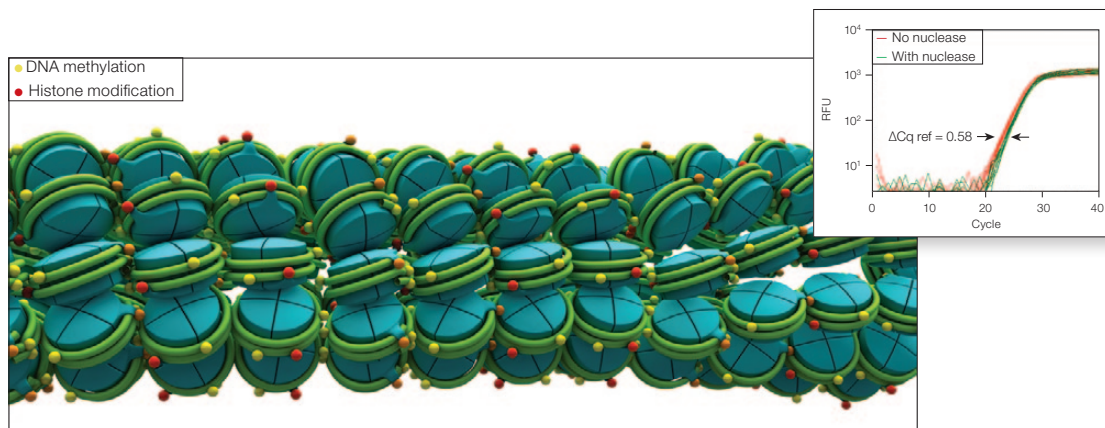
AMPLIFICATION

EpiQ™ Chromatin Analysis Kit

- Rapidly assesses chromatin state in cultured cells
- Generates quantitative chromatin structure information
- Strongly correlates chromatin state with gene expression levels

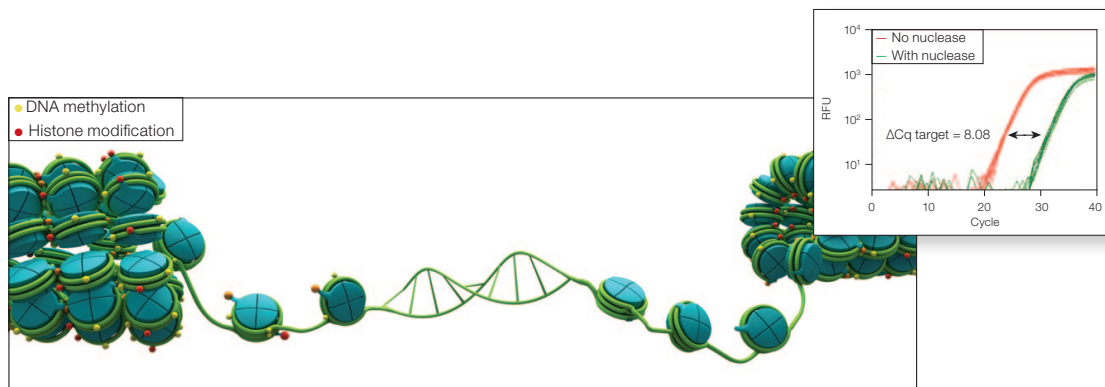
A Novel Research Tool for Epigenetics Analysis

The EpiQ chromatin analysis kit is a real-time PCR assay for the quantitative assessment of chromatin structure and nuclease accessibility.



Heterochromatin

- Transcriptionally silent ▶ inaccessible to transcriptional machinery
- Inaccessible to nuclease ▶ no DNA digestion ▶ available for qPCR ▶ minimal Cq shift



Euchromatin

- Transcriptionally competent ▶ accessible to transcriptional machinery
- Accessible to nuclease ▶ DNA digestion ▶ unavailable for qPCR ▶ large Cq shift



Accelerate Your Epigenetics Research

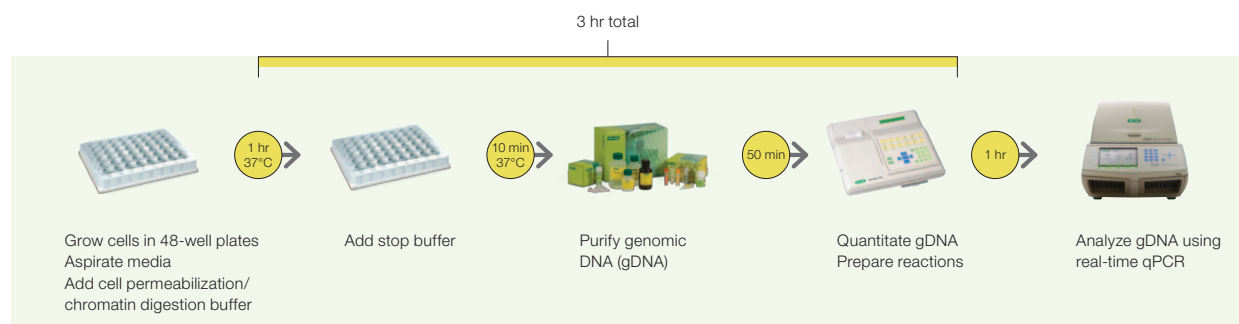
Bio-Rad's EpiQ chromatin analysis kit is a novel epigenetics research tool for the quantitative assessment of chromatin structure in cultured cells. By combining in situ chromatin digestion, genomic DNA purification, and real-time PCR, the chromatin state for several gene promoters can be studied simultaneously. The EpiQ kit helps quantify the impact of epigenetic events, such as DNA methylation and histone modification, on gene expression regulation through chromatin state changes.

- Rapid assessment of chromatin structure in cultured cells (time to results <6 hours)
- Few cells (as little as 5×10^4) required to perform analysis
- Novel assay generates quantitative chromatin structure information with strong correlation to gene expression levels

Epigenetic processes, such as DNA methylation and histone modification, control gene expression by altering chromatin structure. Genes that are actively transcribed are associated with "open" or "accessible" chromatin regions, while genes that

are transcriptionally silent are often in "closed" or "inaccessible" chromatin regions. The EpiQ chromatin analysis kit discriminates between open and closed chromatin regions by employing a nuclease accessibility assay and real-time PCR to quantify the level of accessibility. Rapid results can be obtained in approximately 6 hours and strongly correlate with gene expression levels. The EpiQ kit complements existing epigenetic assays (DNA methylation and chromatin immunoprecipitation), delivering novel insights into mechanisms of gene regulation.

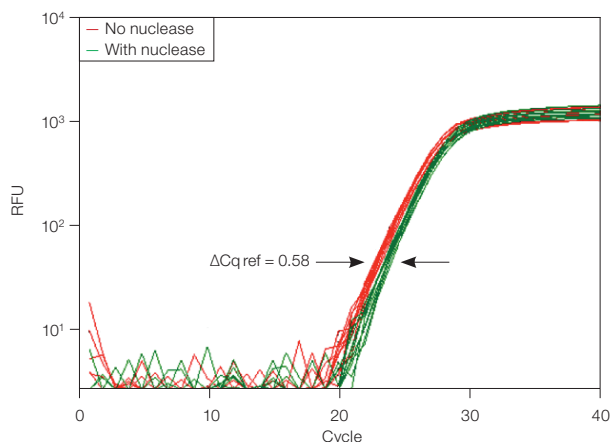
The EpiQ chromatin analysis kit requires cultured (adherent or suspension) cells as starting material and includes the necessary supplies for performing chromatin assessment, including reagents for chromatin digestion, genomic DNA purification, and real-time PCR analysis* using SYBR® Green. The EpiQ kit also includes control qPCR primers for epigenetically silenced (reference) and constitutively expressed (control) gene promoters. Experimental results for user-specified gene targets are analyzed against the reference target to quantify the extent of chromatin accessibility.



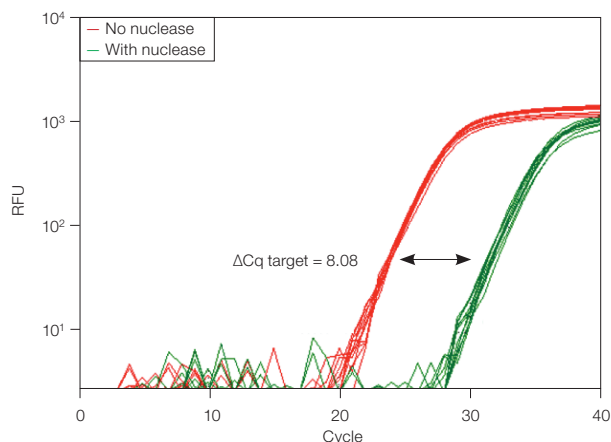
EpiQ chromatin analysis kit workflow. Cells grown in culture plates or flasks are treated for 1 hr at 37°C with chromatin buffer in the presence or absence of nuclease. Chromatin digestion is terminated with stop buffer for 10 min at 37°C, followed by genomic DNA purification and quantification. Chromatin structure is assessed via real-time quantitative PCR by comparing results against an epigenetically silenced (reference) gene.

* The EpiQ™ chromatin SYBR® Green supermix is compatible with all real-time PCR instruments except the ABI 7000, 7300, 7700, and 7900 systems, which require a higher concentration of ROX passive reference dye. ROX passive reference dye can be ordered separately (catalog #172-5858).

A. *HBB* — Reference Gene (epigenetically silenced)

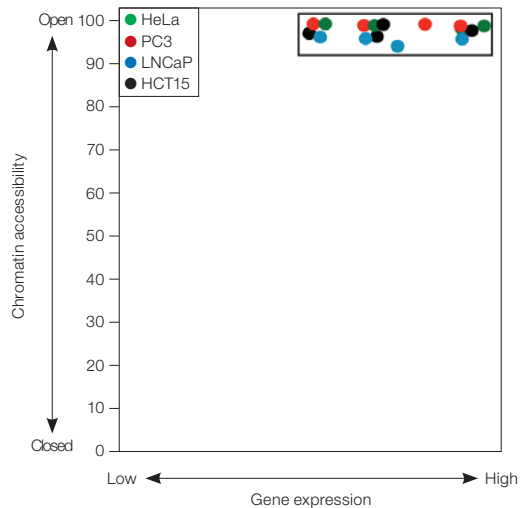


B. *GAPDH* — Target Gene (constitutively expressed)

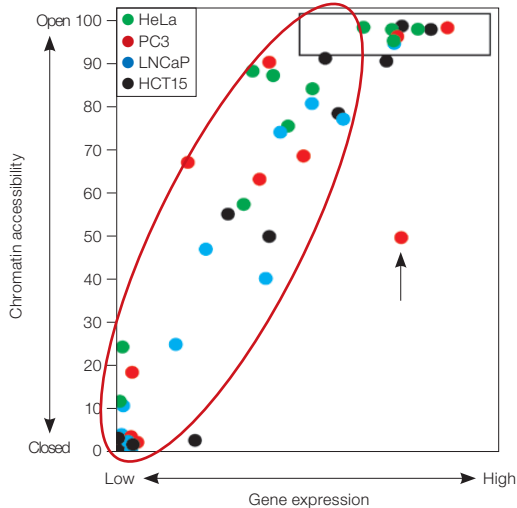


The EpiQ chromatin analysis kit utilizes nuclease accessibility to discriminate open vs. closed chromatin regions. Amplification of proximal promoter regions for the epigenetically silenced *HBB* (reference) gene or the constitutively expressed *GAPDH* (target) gene was carried out in HeLa cells using the EpiQ kit and EpiQ™ chromatin SYBR® Green supermix on the CFX96™ real-time PCR detection system. **A**, closed chromatin regions were protected from nuclease digestion and remained intact prior to amplification, resulting in minimal quantification cycle (Cq) delays ($\Delta Cq = 0.58$) following nuclease treatment; **B**, open chromatin regions were susceptible to nuclease digestion and were unavailable for amplification, leading to significant Cq delays ($\Delta Cq = 8.08$) after nuclease treatment. A comparison of ΔCqs with the amplification efficiencies for each gene target factored in was used to determine the accessibility of the target gene, calculated to be >99% for *GAPDH*. RFU, relative fluorescence units.

A. Constitutively Expressed Genes



B. Epigenetically Regulated Genes



Chromatin structure data from the EpiQ chromatin analysis kit strongly correlate with gene expression levels for several genes in various cell lines. **A**, the chromatin state of the proximal promoter regions for four constitutively expressed genes (*GAPDH*, *ACTB*, *TBP*, and *B2M*) was assessed in four different cell lines (HeLa, PC3, LNCaP, and HCT15). Each gene promoter was determined to be in an open chromatin structure, consistent with medium to high levels of constitutive mRNA expression determined by qPCR. **B**, eleven epigenetically regulated gene promoters (*ABCB1*, *CDH1*, *CDH13*, *DAPK1*, *DKK1*, *GSTP1*, *PTGS2*, *TP73*, *WT1*, *p14ARF*, and *p16INK4A*) were evaluated for chromatin structure in the same four cell lines. Chromatin state varied across the panel of genes and strongly correlated with the gene expression levels. Promoter regions in an open state were associated with medium to high levels of gene expression (shown in the black box, upper right), whereas those in a closed state were linked to low expression (lower left). The EpiQ kit easily identified one outlier gene in PC3 cells (black arrow, ●), which exhibits a rare form of epigenetic regulation — monoallelic methylation — where only one allele is methylated, resulting in 50% chromatin accessibility and high levels of gene expression.

Ordering Information

Catalog #	Description
172-5400	EpiQ Chromatin Analysis Kit , 50 preparations, contains components for chromatin digestion, analysis, and genomic DNA purification, and 2x real-time PCR mix for 500 x 20 µl reactions
172-5401	EpiQ Chromatin Analysis Kit , 100 preparations, contains components for chromatin digestion, analysis, and genomic DNA purification, and 2x real-time PCR mix for 1,000 x 20 µl reactions
172-5402	EpiQ Chromatin Preparation Kit , 50 preparations, contains components for chromatin digestion, analysis, and genomic DNA purification prior to real-time PCR analysis
172-5403	EpiQ Chromatin Preparation Kit , 100 preparations
172-5404	EpiQ™ Chromatin SYBR® Green Supermix , 5 ml, 2x real-time PCR mix, contains dNTPs, iTaq™ DNA polymerase, MgCl ₂ , SYBR® Green I, ROX passive reference dye, fluorescein, stabilizers, for 500 x 20 µl reactions
172-5405	EpiQ™ Chromatin SYBR® Green Supermix , 10 ml, for 1,000 x 20 µl reactions

ROX is a trademark of Applied Biosystems Corporation. SYBR is a trademark of Molecular Probes, Inc. Bio-Rad Laboratories, Inc. is licensed by Molecular Probes, Inc. to sell reagents containing SYBR Green I for use in real-time PCR, for research purposes only.

Bio-Rad's real-time thermal cyclers are licensed real-time thermal cyclers under Applied Biosystems' United States Patent Number 6,814,934 B1 for use in research, human in vitro diagnostics, and all other fields except veterinary diagnostics.

Bio-Rad's real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Applied Biosystems AG: U.S. Patent Numbers 6,767,512 and 7,074,367.

Purchase of EpiQ chromatin SYBR Green supermix includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research. No other patent rights are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.



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