SYBR® Green Cells-to-CT™ Control Kit

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Store at –20°C.
Do not store in a frost-free freezer.

Catalog # (P/N):	4402959
Product Description:	The SYBR® Green Cells-to-CT™ Control Kit is a set of reagents designed for use with SYBR Green Cells-to-CT Kits as reverse transcription and PCR controls.
Components:	100 µL Xeno™ RNA Control, 10^5 copies/µL 250 µL 20X SYBR Xeno Primers 250 µL 20X SYBR ACTB Primers
Amount:	Xeno RNA Control: approximately 100 lysis reactions SYBR Xeno and SYBR ACTB Primers: up to 250 PCRs (20 µL) each
Storage Conditions:	Store at –20°C. ***Do not store in a frost-free freezer.***
Safety Information:	Read the Safety Data Sheet, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

USER INFORMATION

General Information:

The *Power* SYBR® Green Cells-to-CT™ and Fast SYBR® Green Cells-to-CT™ Kits enable reverse transcription and SYBR Green-based, real-time PCR (real-time RT-PCR) analysis of 10–10^5 cultured cells, without purifying RNA. These kits include reagents for cell lysis, reverse transcription, and SYBR Green-based real-time PCR (standard or fast cycling conditions, respectively) with user-supplied primer sets targeting genes of interest.

The SYBR Green Cells-to-CT Control Kit is designed for use with the *Power* SYBR Green and Fast SYBR Green Cells-to-CT Kits. It includes Xeno RNA Control, a synthetic RNA transcript with a unique sequence that lacks homology to current annotated biological sequences, and a PCR primer set for the Xeno RNA Control target. It also includes a PCR primer set for the highly expressed endogenous control gene β-Actin (ACTB). Together these reagents provide positive controls for reverse transcription and SYBR Green-based real-time PCR, and can indicate the presence of any reverse transcription or PCR inhibitors.

Real-time RT-PCR with SYBR ACTB Primers can also provide an endogenous control for sample normalization, as well as to confirm sufficient cell input in experiments with samples consisting of <100 cells per lysis.

Handling Instructions:

RNA is very sensitive to degradation by exogenous ribonucleases introduced during handling. Wear gloves when handling this product. Use RNase-free reagents, tubes, and barrier pipette tips.

Thawing Instructions for Xeno RNA Control
Thaw just to completion at 37°C, vortex for a few seconds when fully thawed, and place on ice. Aliquot the RNA, if necessary to minimize freeze-thaw cycles (≤5).

Applications:

Controls for the *Power* SYBR Green and Fast SYBR Green Cells-to-CT Kits
The SYBR Green Cells-to-CT Control Kit is designed for use with SYBR Green-based Cells-to-CT Kits. Refer to the protocol for the appropriate kit for detailed instructions, available under the "Literature/Resources" tab at its web catalog page (search by the kit part number at www.appliedbiosystems.com).

Positive Control for Real-Time RT-PCR, Determining Optimal Cell Number Range for Lysis, and Detection of RT-PCR Inhibitors
Perform the pilot experiment described in the Appendix of the *Power* SYBR Green or Fast SYBR Green Cells-to-CT Kit protocols (P/N 4403787 and 4403786, respectively). In this experiment, cells are serially diluted and lysed in Lysis Solution. Xeno RNA Control is added to the Stop Solution that is mixed into the lysates to inactivate the lysis reagents. The lysates are then subjected to SYBR Green-based real-time RT-PCR targeting Xeno RNA and β-Actin, using the primer sets included in the SYBR Green Cells-to-CT Control Kit.

Expected Results for Xeno RNA Control: When used as described (1 µL Xeno RNA Control per 50 µL lysis, 10 µL of lysate in each 50 µL RT reaction, and 4 µL of each RT reaction per 20 µL PCR reaction) the C_T values from the Xeno RNA Control should be consistent (±1 C_T) regardless of the number of cells in the lysis reaction, indicating that no RT-PCR inhibitors are present in the Cells-to-CT lysate. An increase in C_T values with increasing cells/lysis reaction indicates the presence of inhibitors of RT-PCR. In future experiments, use cell numbers that do not cause an increase in C_T value.

Using the above conditions and the Applied Biosystems GeneAmp® PCR System 9700 for reverse transcription and the 7900HT Fast Real-Time PCR System, C_T values of 28–30 are typically seen. However, the consistency of C_T values is much more important than the absolute values, which can vary among instrument platforms.

Expected Results for β-Actin: Create a plot of C_T versus the log of the number of cells in the lysis. The C_T values should decrease in a linear fashion as the number of cells increase, for cell numbers that are compatible with the procedure. At cell concentrations that result in incomplete lysis or RT-PCR inhibition, the data will no longer be linear. In future experiments, do not exceed the number of cells per lysis reaction that provided results within the linear range in the pilot experiment.

Endogenous Control for Samples with Low Cell Number
When using the *Power* SYBR Green or Fast SYBR Green Cells-to-CT Kits, confirm the presence of cells in samples containing <100 cells per lysis using the SYBR ACTB Primers supplied with the SYBR Green Cells-to-CT Control Kit. Using the conditions described in the appropriate SYBR Green Cells-to-CT Kit protocol, C_T values < 35 indicate the presence of cells in those samples.

Endogenous Control for Normalization of SYBR Green-based Real-time RT-PCR
Use the SYBR ACTB Primers supplied with this kit as an endogenous control for signal normalization of SYBR Green-based real-time RT-PCR. For further information on data analysis of real-time PCR, see the Applied Biosystems *Real-Time PCR Systems Chemistry Guide* (P/N 4348358).

RELATED PRODUCTS

***Power* SYBR® Green Cells-to-CT™ Kit**
P/N 4402953, 4402954, 4402955
Optimized lysis, reverse transcription, and real-time PCR reagents that enable two-step, SYBR Green-based, real-time RT-PCR analysis directly from cultured cells, using standard cycling conditions.

Fast SYBR® Green Cells-to-CT™ Kit
P/N 4402956, 4402957
Optimized lysis, reverse transcription, and real-time PCR reagents that enable two-step, SYBR Green-based, real-time RT-PCR analysis directly from cultured cells, using fast cycling conditions.

QUALITY CONTROL

Functional Testing:	SYBR Xeno Primers and SYBR ACTB Primers are tested functionally in real-time RT-PCR using Xeno RNA Control or HeLa S3 Total RNA as template. In addition, relevant kit components are tested in the following nuclease assays.
Nonspecific Endonuclease Activity:	A sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.
Exonuclease Activity:	A sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.
RNase Activity:	A sample is incubated with labeled RNA, followed by PAGE analysis.

OTHER INFORMATION

Safety Data Sheets:
Safety Data Sheets (SDSs) are available from: **www.invitrogen.com/sds** or **www.appliedbiosystems.com/sds**. **Note:** For the SDSs of chemicals not distributed by Life Technologies, **contact the chemical manufacturer**.

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