

# TaqMan® Cells-to-CT™ Control Kit

Store at –20°C.

*Do not store in a frost-free freezer.*

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| <b>Catalog #:</b>           | 4386995  |
| <b>Product Description:</b> | The TaqMan® Cells-to-CT™ Control Kit is a set of reagents designed for use with the TaqMan Cells-to-CT Kits as reverse transcription and PCR controls.   |
| <b>Components:</b>          | 100 µL XenoRNA™ Control, 10 <sup>5</sup> copies/µL<br>250 µL 20X XenoRNA™ Control TaqMan® Gene Expression Assay (FAM™/MGB, assay ID Ac00010014_a1)<br>250 µL 20X ACTB TaqMan® Gene Expression Assay (FAM™/MGB, assay ID Hs03023880_g1) |
| <b>Amount:</b>              | XenoRNA Control: approximately 100 lysis reactions<br>XenoRNA Control and ACTB TaqMan Gene Expression Assays: up to 250 PCRs (20 µL) each  |
| <b>Storage Conditions:</b>  | Store at –20°C. <i>Do not store in a frost-free freezer.</i>   |

## USER INFORMATION

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| <b>General Information:</b>       | <p>The TaqMan® Gene Expression Cells-to-CT™ Kit (Cat #AM1728, AM1729) enables reverse transcription and real-time PCR (real-time RT-PCR) analysis of lysates from 10–10<sup>5</sup> cultured cells without purifying RNA. It includes reagents for cell lysis, reverse transcription, and real-time PCR with user-supplied TaqMan Gene Expression Assays targeting genes of interest.</p> <p>The TaqMan Cells-to-CT Control Kit is designed for use with the TaqMan Cells-to-CT Kits. It includes XenoRNA™ Control, a synthetic RNA transcript with a unique sequence that lacks homology to current annotated biological sequences, and a TaqMan Gene Expression Assay for the XenoRNA Control target. It also includes a TaqMan Gene Expression Assay (ACTB) for the highly expressed endogenous control gene β-Actin. Together these reagents provide positive controls for reverse transcription and real-time PCR, and can indicate the presence of any reverse transcription or PCR inhibitors.</p> <p>The ACTB TaqMan Gene Expression Assay can also be used as an endogenous control for sample normalization, as well as to confirm sufficient cell input in experiments with samples consisting of &lt;100 cells per lysis.</p> <p>The TaqMan Gene Expression Assays included in the TaqMan Cells-to-CT Control Kit are not primer limited and are not intended for multiplex real-time RT-PCR. When using these control TaqMan Gene Expression Assays, perform PCRs in parallel with those for your gene of interest.</p>  |
| <b>RNA Handling Instructions:</b> | <p>RNA is very sensitive to degradation by exogenous ribonucleases introduced during handling. Wear gloves when handling RNA components. Use RNase-free reagents, tubes, and barrier pipette tips.</p> <p><b>Thawing Instructions for RNA</b><br/>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).</p>  |
| <b>Applications:</b>              | <p><b>Controls for the TaqMan Gene Expression Cells-to-CT Kit</b><br/>The TaqMan Cells-to-CT Control Kit is designed for use with TaqMan Cells-to-CT Kits. Refer to any of the TaqMan Cells-to-CT Kit Instruction Manuals for the detailed protocol, available at:<br/><a href="http://www.ambion.com/techlib/prot/fm_AM1728.pdf">www.ambion.com/techlib/prot/fm_AM1728.pdf</a></p> <p><b>Positive Control for Real-Time RT-PCR, Determining Optimal Cell Number Range for Lysis, and Detection of RT-PCR Inhibitors</b><br/>Perform the pilot experiment described in the Appendix of the TaqMan Cells-to-CT Kit Instruction Manuals. In this experiment, cells are serially diluted and lysed in Lysis Solution. XenoRNA Control is added to the Stop Solution that is mixed into the lysates to inactivate the lysis reagents. The lysates are then subjected to real-time RT-PCR targeting XenoRNA and β-Actin using the TaqMan Gene Expression Assays included in the TaqMan Cells-to-CT Control Kit.</p> <p><b>Expected Results for XenoRNA Control:</b> When used as described (1 µL XenoRNA 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<sub>t</sub> values from the XenoRNA Control should be consistent (±1 C<sub>t</sub>) 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<sub>t</sub> 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<sub>t</sub> value.</p> <p>Using the above conditions and the Applied Biosystems GeneAmp® PCR System 9700 for reverse transcription and the 7900HT Fast Real-Time PCR System, C<sub>t</sub> values of 28–29 are typically seen. However, the consistency of C<sub>t</sub> values is much more important than the absolute values, which can vary among instrument platforms.</p> |

**Expected Results for  $\beta$ -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 TaqMan Gene Expression Cells-to-CT Kit, confirm the presence of cells in samples containing <100 cells per lysis using the ACTB TaqMan Gene Expression Assay supplied with the TaqMan Cells-to-CT Control Kit. Using the conditions described in the TaqMan Gene Expression Cells-to-CT Kit Instruction Manual,  $C_T$  values < 35 indicate the presence of cells in those samples.

**Endogenous Control for Normalization of Real-time RT-PCR**

Use the ACTB TaqMan Gene Expression Assay supplied with this kit as an endogenous control for signal normalization of real-time RT-PCR using TaqMan Gene Expression Assays. The ACTB TaqMan Gene Expression Assay is not primer limited and is not intended for multiplexing; perform PCR in parallel with a TaqMan Gene Expression Assay for your target of interest. For further information on data analysis of real-time PCR, see *Real-Time PCR Systems Chemistry Guide* (Part #4348358).

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**RELATED PRODUCTS**

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**TaqMan<sup>®</sup> Gene Expression Cells-to-CT™ Kit**

Cat #AM1728, AM1729

A robust set of lysis, reverse transcription, and PCR reagents that enables streamlined real-time RT-PCR analysis of cultured-cell lysates with user-supplied TaqMan<sup>®</sup> Gene Expression Assays.

**TaqMan<sup>®</sup> Gene Expression Assays**

[www.allgenes.com](http://www.allgenes.com)

A comprehensive collection of over 700,000 probe and primer sets for quantitative gene expression analysis using real-time PCR.

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**QUALITY CONTROL**

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**Functional Testing:**

XenoRNA™ Control TaqMan<sup>®</sup> Gene Expression Assay and ACTB TaqMan<sup>®</sup> Gene Expression Assay are tested functionally in one-step real-time RT-PCR using XenoRNA Control or HeLa S3 Total RNA as template.

In addition, XenoRNA Control undergoes the following rigorous nuclease testing.

**Nonspecific Endonuclease Activity:**

Meets or exceeds specification when a sample is incubated for 14–16 hr with 300 ng supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

**Exonuclease Activity:**

Meets or exceeds specification when a sample is incubated for 14–16 hr with 40 ng <sup>32</sup>P-labeled *Sau3A* fragments of pUC19 and analyzed by PAGE.

**RNase Activity:**

Meets or exceeds specification when a sample is incubated for 14–16 hr with 25 ng <sup>32</sup>P-labeled RNA and analyzed by PAGE.

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**OTHER INFORMATION**

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**Material Safety Data Sheets:**

Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: [www.ambion.com/techlib/msds](http://www.ambion.com/techlib/msds). Alternatively, e-mail your request to [MSDS\\_Inquiry\\_CCRM@appliedbiosystems.com](mailto:MSDS_Inquiry_CCRM@appliedbiosystems.com). Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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