

sample prep

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invitrogen

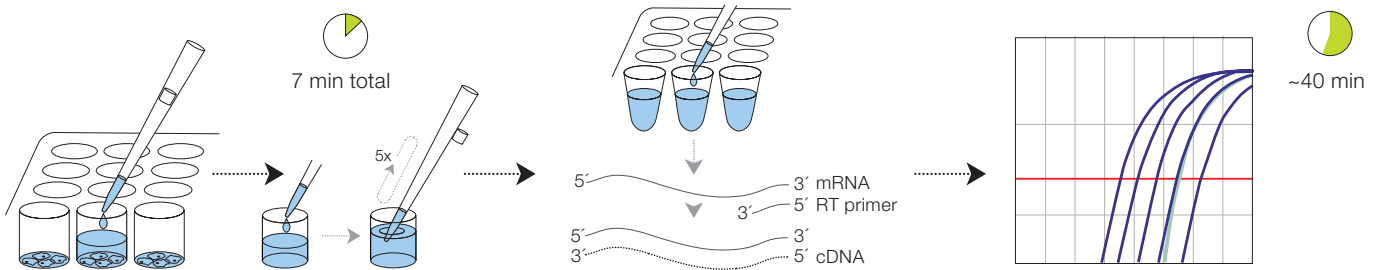


Accelerate gene expression without RNA purification

Fast Advanced Cells-to-C_T kits—a faster and more sensitive way to measure gene expression

ThermoFisher
SCIENTIFIC

Gene expression experimental workflow



Cell Lysis + Stop Solution

1. Remove culture medium. Wash cells with PBS.
2. (Optional) Dilute DNase into Lysis Solution.
3. Add Lysis Solution and mix 5 times.
4. Incubate for 5 min at room temp (19–25°C).
5. Add Stop Solution and mix 5 times.
6. Incubate for 2 min at room temp.

Reverse transcription

1. Assemble an RT master mix and aliquot into reaction tubes or plates.
2. Add lysate (up to 45%) and mix thoroughly.
3. Run the RT thermal cycler program.

qPCR: TaqMan or SYBR Green Assay

1. Assemble a PCR cocktail and aliquot into reaction tubes or plates.
2. Add cDNA and mix thoroughly.
3. Run the reactions in a qPCR instrument.

2-step workflow

Step 1

Step 2

Figure 1. The procedure for Fast Advanced Cells-to-C_T kits is a 2-step process that involves reverse transcription and qPCR following cell lysis. A 1-step reaction is when RT and qPCR are performed in a single well. In the qPCR step, TaqMan Assays use custom primer and probe combinations designed for each target. These assays are more sensitive and specific than SYBR Green assays, which use generic qPCR primers.

New Invitrogen™ Fast Advanced Cells-to-C_T™ kits offer researchers the ability to skip RNA purification entirely by going straight from cultured cell samples to measuring relative gene expression by real-time RT-PCR (RT-qPCR). And for flexibility, Cells-to-C_T kits are available in both TaqMan® and SYBR™ Green formats.

These kits provide a complete workflow for RT-qPCR analysis directly from cultured cells without RNA purification. Featuring a unique method for lysing cultured cells while removing genomic DNA (gDNA) and preserving RNA integrity, the kits contain reverse transcription (RT) reagents for cDNA synthesis and either TaqMan or SYBR Green master mixes for real-time PCR analysis.

Two formats available for increased flexibility

The new TaqMan Fast Advanced Cells-to-C_T Kit utilizes an improved reverse transcriptase enzyme that is more sensitive to detect abundant or rare transcripts. This makes it possible to perform expression analysis directly from cultured cells without RNA purification. This kit helps save time and offers a simple workflow that is suitable for a few samples, but can also be easily incorporated into automated, high-throughput applications.

The TaqMan Fast Advanced Cells-to-C_T Kit offers:

- The same patented Lysis Solution and Stop Solution as the original TaqMan Gene Expression Cells-to-C_T Kit
- Superior specificity for detecting targets
- Extraordinary ease and speed—96 samples for RT-qPCR in typically <10 min
- No tedious RNA purification—no columns, heating, centrifugation, or sample transfer
- Superior performance—designed for consistent accuracy, reproducibility, and sensitivity with 10–100,000 cells
- Premium validated kit workflows—complete sets of reagents preoptimized to work efficiently right out of the box
- Detect virtually any gene product with more than 2 million predesigned assays
- Affordable assays for nearly every human, mouse, and rat gene in the RefSeq database
- Available for 28 species and some pathogens

The new SYBR Green Fast Advanced Cells-to-C_T Kit is a fast, easy, and robust method to go directly from cultured cells to real-time PCR results. This proven cell lysis and RNA stabilization technology eliminates the laborious and time-consuming RNA purification process completely. The kit is powered by Applied Biosystems™ AmpliTaq Gold™ DNA Polymerase, LD (Low DNA), which provides the highest levels of specificity with standard real-time PCR.

The SYBR Green Fast Advanced Cells-to-C_T Kit offers:

- The same patented Lysis Solution and Stop Solution as the original TaqMan Gene Expression Cells-to-C_T Kit
- High specificity for detecting targets
- Extraordinary ease and speed—96 samples for RT-qPCR in typically <10 min
- No tedious RNA purification—no columns, heating, or centrifugation
- Superior performance—designed for consistent accuracy, reproducibility, and sensitivity with 10–100,000 cells
- Productive validated kit workflows—complete sets of reagents preoptimized to work efficiently right out of the box

Higher sensitivity in less time

The Cells-to-C_T Stop Solution allows for greater sample input in the downstream RT and qPCR reactions, greatly increasing the sensitivity for detecting rare targets. Both TaqMan and SYBR Green Fast Advanced Cells-to-C_T Kits use the Stop Solution, providing excellent sensitivity with a very fast workflow. These kits are approximately 48 times faster than most high-throughput RNA purification kits. Fast Advanced Cells-to-C_T kits can process 96 or 384 wells at once; the experimental workflow only takes about 10 minutes for a 96- or 384-well plate. Products from other suppliers use low pH and dilution in their workflows, which restricts how much cell lysate can be used in downstream steps.

Gene expression experimental workflow: comparison

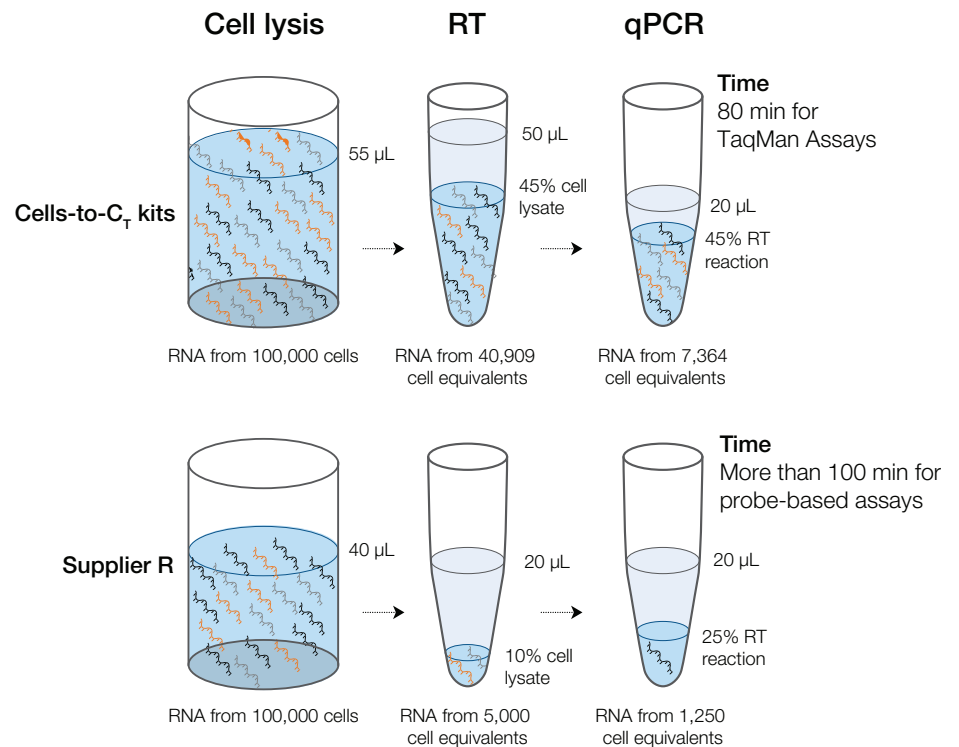


Figure 2. Volume of lysate or RT reaction at each step in the workflow.

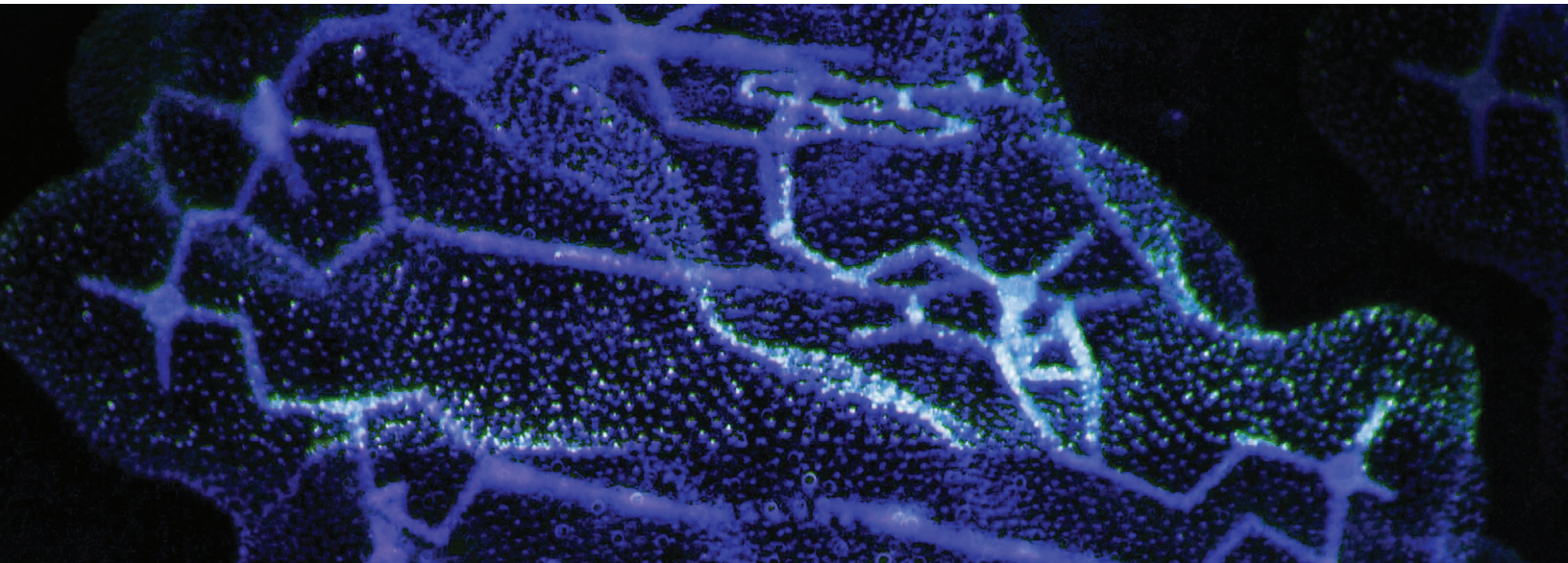


Table 1. Which real-time chemistry is right for you?

	Cells-to-C _T kits (1-step)		Fast Advanced Cells-to-C _T kits (2-step)	
One complete workflow	Lysis → RT → qPCR			
Cells-to-C _T Lysis, DNase, and Stop Solutions	Yes			
Detection chemistry	SYBR Green format*	TaqMan format**	SYBR Green format*	TaqMan format**
Time to results	120 min	120 min	95 min	80 min
Working range	10–100,000 cells	10–100,000 cells	10–100,000 cells	10–100,000 cells
Specificity	Medium†	High	Medium†	High
Sensitivity (maximum percentage of sample that makes it into the qPCR reaction)	10% lysate	10% lysate	13.5% lysate	20% lysate
Reproducibility	Medium†	High	Medium†	High
Multiplexing		✓		✓
Predesigned assays		✓		✓
Typically requires user design, experimental optimization	✓		✓	
Gene expression	Low level of quantitation	High level of quantitation	Low level of quantitation	High level of quantitation
Applications	<ul style="list-style-type: none"> • Gene expression • DNA quantitation (pathogen detection) • CHiP 	<ul style="list-style-type: none"> • Gene expression • DNA quantitation • CHiP • SNP genotyping • Copy number variation • Pathway analysis • microRNA and small RNAs • Mutation detection • Multiplexing 	<ul style="list-style-type: none"> • Gene expression • DNA quantitation • CHiP • SNP genotyping • Copy number variation • Pathway analysis • microRNA and small RNAs • Mutation detection • Multiplexing 	<ul style="list-style-type: none"> • Gene expression • DNA quantitation • CHiP • SNP genotyping • Copy number variation • Pathway analysis • microRNA and small RNAs • Mutation detection • Multiplexing
Reagents	<ul style="list-style-type: none"> • SYBR Green primers • SYBR Green master mixes 	<ul style="list-style-type: none"> • TaqMan Assays • TaqMan master mixes 	<ul style="list-style-type: none"> • SYBR Green primers • SYBR Green master mixes 	<ul style="list-style-type: none"> • TaqMan Assays • TaqMan master mixes

* SYBR Green–based detection uses SYBR Green dye (a dsDNA-binding dye) to detect PCR product as it accumulates during PCR.

** TaqMan-based detection uses a fluorogenic probe specific to the target gene to detect the target as it accumulates during PCR.

† SYBR Green–based assays require primers to be designed and validated to ensure specificity. Specificity and reproducibility depend on the template quality and primer design and optimization.

Selecting the appropriate kit

Choose a 1-step kit if you:

- Have many samples with one or a few targets
- Do not store cDNA
- Use liquid handling robotics

Choose a 2-step kit if:

- You need higher sensitivity for rare transcripts
- Archiving of cDNA is possible
- Multiple targets will be quantified from cDNA
- You use liquid handling robotics

Skip RNA purification without compromising performance

Fast Advanced Cells-to-C_T 2-step kits are faster and more convenient to use than traditional RNA purification without compromising sensitivity. Because the workflow is lysate-based, there is no loss of RNA with Cells-to-C_T kits. In traditional RNA purification, a small amount of RNA is always lost to incomplete elution off of filters or magnetic beads. Thus, the ability to detect rare RNAs in

low input cell samples (10–1,000) is higher when samples are processed with Cells-to-C_T kits compared to results from the same samples using RNA purification workflows. This, coupled with the benefits of the Stop Solution, gives Cells-to-C_T workflows more sensitivity than competitor kits to detect both abundant and low copy transcripts.

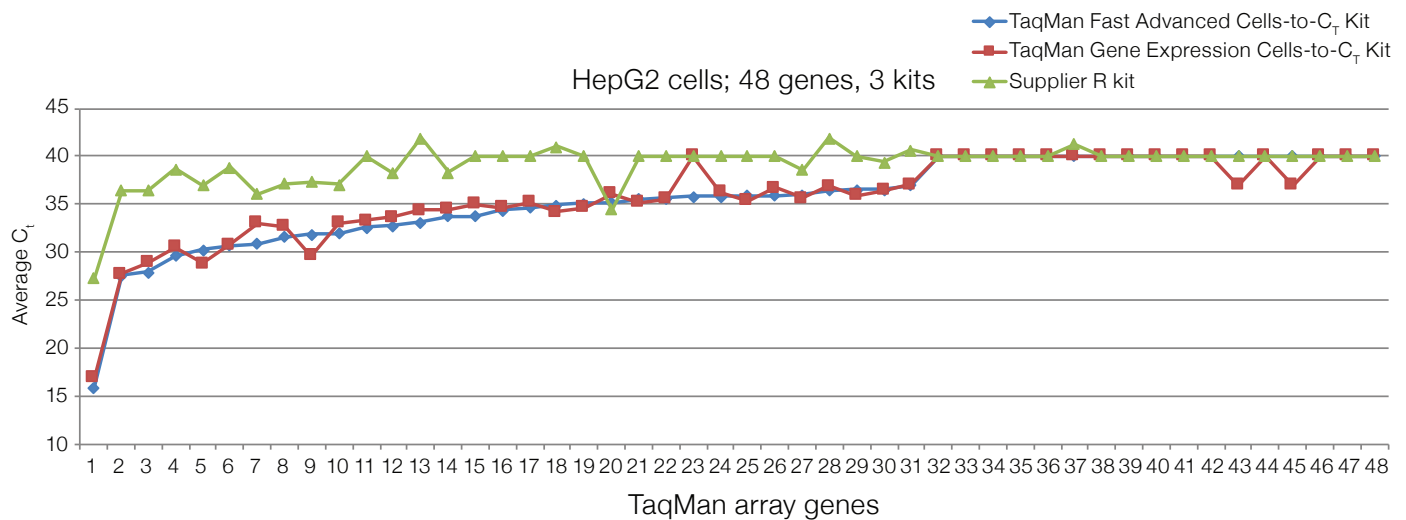
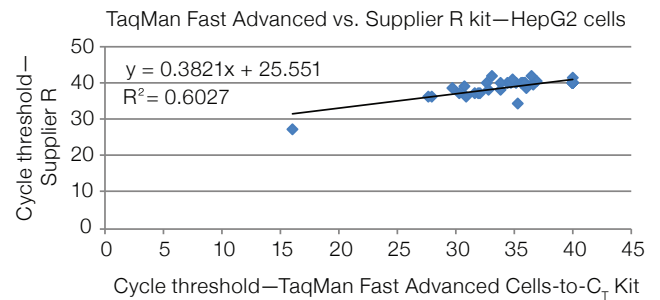
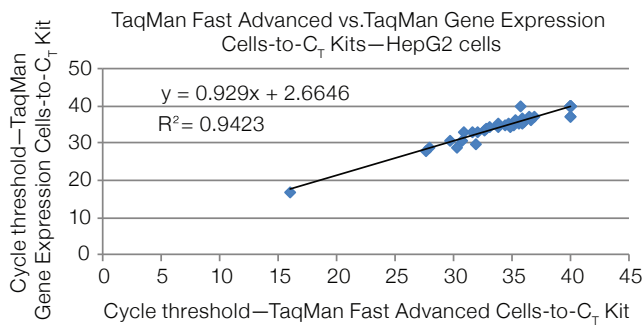


Figure 3. Sensitivity compared to other kits. 10⁴ cells were lysed following each kit's protocol. The maximum amount of lysate was added to each kit's RT (45% for Cells-to-C_T and Fast Advanced Cells-to-C_T kits; 10% for other supplier). Then 25% of RT was added into the qPCR reactions of a TaqMan Array for phosphodiesterases, using each kit's qPCR master mix. The Fast Advanced Cells-to-C_T kit performed on average 0.5 C_T better than the original Cells-to-C_T kit and 3.6 C_T better than another supplier's kit (11x more sensitive than other supplier).

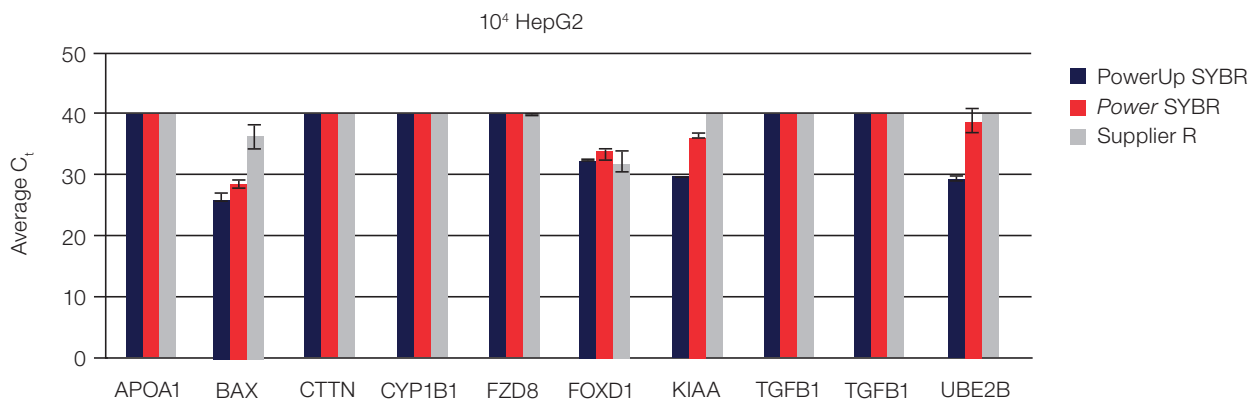


Figure 4. Comparison of detection. 10⁴ HepG2 cells were lysed following each kit's protocol. The maximum amount of lysate was added to each kit's RT (45% for Cells-to-C_T and Fast Advanced Cells-to-C_T kits; 10% for other supplier). Then 25% of RT was added into the qPCR reactions for the other supplier's kit; 30% was added to the qPCR reaction for the Fast Advanced Cells-to-C_T kit, 45% added to *Power* SYBR kit. Assayed 10 different genes. The majority of genes were not detected in this cell line. Of the genes that were detected, the Fast Advanced Cells-to-C_T kit has lower C_T values than the other kits for most genes.

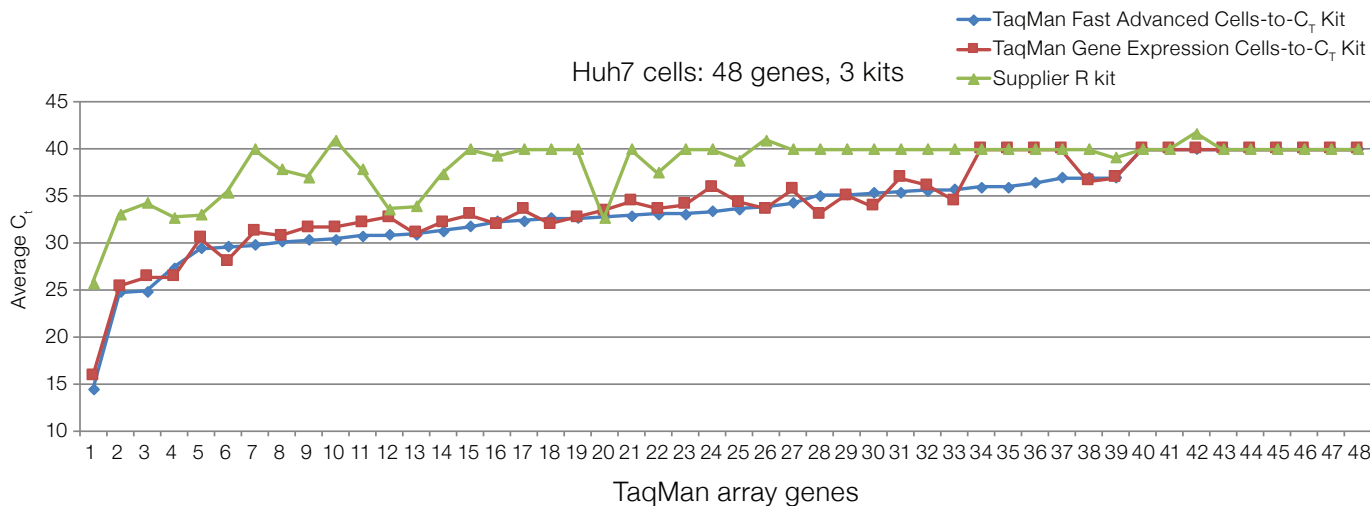
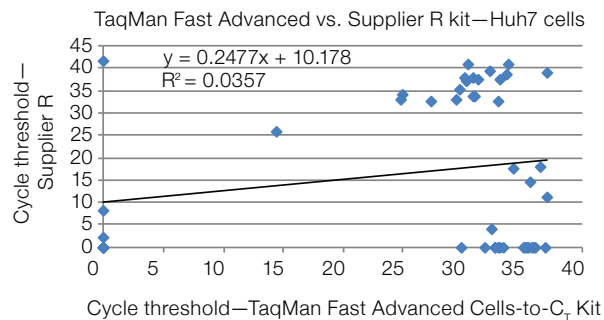
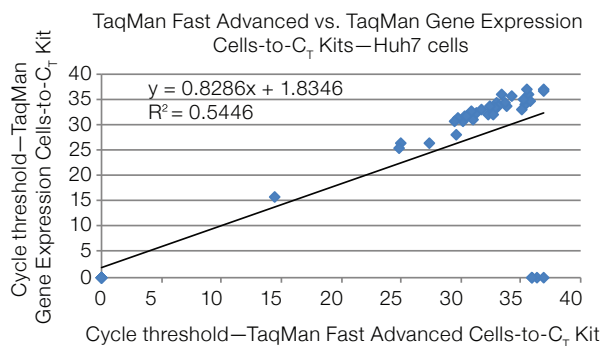


Figure 5. 10⁴ cells were lysed following each kit's protocol. The maximum amount of lysate was added to each kit's RT (45% for the original Cells-to-C_T kit and Fast Advanced Cells-to-C_T kit; 10% for the other supplier's kit). Then 25% of RT was added into the qPCR reactions of a TaqMan Array for phosphodiesterases, using each kit's qPCR master mix. Conclusions: The Fast Advanced Cells-to-C_T kit performs on average 0.7 C_T better than the original Cells-to-C_T kit and 4.7 C_T better than the other supplier's kit. This means that the Fast Advanced kit is 14x more sensitive than the other supplier's kit.

Find out how your Cells-to-C_T workflow can be automated at thermofisher.com/autocellstoc

Real-time PCR applications for a complete experimental workflow solution

To help you experience the power of optimized results across diverse applications, we also offer real-time PCR instrumentation solutions to complete your experimental workflow. Real-time PCR is used for sensitive, specific detection and quantification of nucleic acid targets.

We have developed powerful assay design algorithms, optimized master mixes, intuitive data analysis software,


and flexible instrumentation to help harness the power of qPCR across a rich and diverse set of applications. Discover solutions for your qPCR-based research.

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Which instrument fits your needs?

QuantStudio real-time PCR and digital PCR systems

For when you need:	Ultimate simplicity	Total control	Room to grow	More versatility	Maximum productivity
	QuantStudio 3 System	QuantStudio 5 System	QuantStudio 6 Flex System	QuantStudio 7 Flex System	QuantStudio 12K Flex System
Real-time PCR					
					
Colors	4 colors	5 or 6 colors (21 filter combinations)	5 colors	6 colors (21 filter combinations)	6 colors (21 filter combinations)
Available formats*	96-well (0.2 mL block) 96-well Fast (0.1 mL block)	96-well (0.2 mL block) 96-well Fast (0.1 mL block) 384-well	96-well 96-well Fast 384-well	96-well 96-well Fast 384-well TaqMan Array card (384-well microfluidic card)	96-well 96-well Fast 384-well TaqMan Array card (384-well microfluidic card) OpenArray plates (3,072 through-holes)
Dimensions	27 x 50 x 40 cm	27 x 50 x 40 cm	90.7 x 74.7 x 12.5 cm	90.7 x 74.7 x 12.5 cm	90.7 x 74.7 x 12.5 cm
Block change	Fixed	Fixed	Block change from front in less than 1 min; no tools required		
VeriFlex temperature control	3 zones	6 zones (96-well blocks only)	N/A	N/A	N/A
Automation-compatible	No	No	No	Yes	Yes
Throughput	Medium	Medium	Medium	High	Very high
21 CFR Part 11–enablement	Security	Security, auditing, e-signature package	Optional security, auditing, e-signature packages available		
Touch screen	Yes, interactive	Yes, interactive	Yes	Yes	Yes
Key applications	<ul style="list-style-type: none"> Gene expression miRNA profiling SNP genotyping Copy number variation Protein thermal shift High resolution melt Pathogen detection 	<ul style="list-style-type: none"> Gene expression miRNA profiling SNP genotyping Copy number variation Protein thermal shift High resolution melt Pathogen detection 	<ul style="list-style-type: none"> Gene expression miRNA profiling SNP genotyping Copy number variation Protein thermal shift High resolution melt Pathogen detection 	<ul style="list-style-type: none"> Gene expression miRNA profiling SNP genotyping Copy number variation Protein thermal shift High resolution melt Pathogen detection Pharmacogenomics 	<ul style="list-style-type: none"> Gene expression miRNA profiling SNP genotyping Copy number variation Protein thermal shift High resolution melt Pathogen detection Pharmacogenomics Growing menu of qualified solutions

Some instruments are also available in a diagnostic format. Learn more at thermofisher.com/qsdx

 = cloud-enabled instrument

Ordering information

Product	Quantity	Cat. No.
TaqMan Fast Advanced Cells-to-C _T Kit	40 reactions	A35374
	100 reactions	A35377
	400 reactions	A35378
SYBR Green Fast Advanced Cells-to-C _T Kit	40 reactions	A35379
	100 reactions	A35380
	400 reactions	A35381
Fast Advanced Cells-to-C _T bulk RT reagents	2,500 reactions	A39110
Cells-to-C _T bulk lysis reagents	2,500 reactions	4391851C
Cells-to-C _T 1-Step TaqMan Kit	20 reactions	A25605
	100 reactions	A25603
	400 reactions	A25602
TaqMan Gene Expression Cells-to-C _T Kit	40 reactions	4399002
	100 reactions	AM1728
	400 reactions	AM1729
TaqMan MicroRNA Cells-to-C _T Kit	100 reactions	4391848
	20 reactions	A25601
Cells-to-C _T 1-Step <i>Power</i> SYBR Green Kit	100 reactions	A25600
	400 reactions	A25599
	40 reactions	4402953
<i>Power</i> SYBR Green Cells-to-C _T Kit	100 reactions	4402954
	400 reactions	4402955
Single Cell-to-C _T RT-qPCR Kit	50 reactions	4458237
	400 reactions	4458236
Cells-to-C _T Stop Solution	1 mL	4402960
Stabilized Blood-to-C _T Nucleic Acid Preparation Kit for qPCR, compatible with PAXgene or Tempus Blood RNA Tubes	50 reactions	4449079
Stabilized Blood-to-C _T Nucleic Acid Preparation Kit for qPCR, compatible with PAXgene Blood RNA Tubes	200 reactions	4449082
Stabilized Blood-to-C _T Nucleic Acid Preparation Kit for qPCR, compatible with Tempus Blood RNA Tubes	200 reactions	4449080
SYBR Green Cells-to-C _T Control Kit	100 reactions	4402959
QuantStudio 3 Real-Time PCR System, 96-well, 0.2 mL, desktop	1 instrument	A28572
QuantStudio 5 Real-Time PCR System, 96-well, 0.2 mL, desktop	1 instrument	A28574
QuantStudio 6 Flex Real-Time PCR System, 96-well Fast, desktop	1 instrument	4485697
QuantStudio 7 Flex Real-Time PCR System, 96-well Fast, desktop	1 instrument	4485693
QuantStudio 12K Flex Real-Time PCR System, Fast 96-well block, desktop	1 instrument	4471088
TaqMan Fast Advanced Master Mix	1 x 5 mL	4444557
PowerUp SYBR Green Master Mix	5 mL	A25742

Find out more at thermofisher.com/cellstoc

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