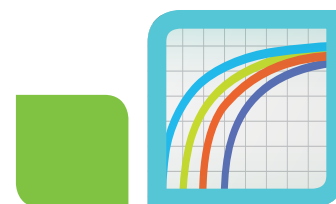


# Want to Skip RNA Isolation?

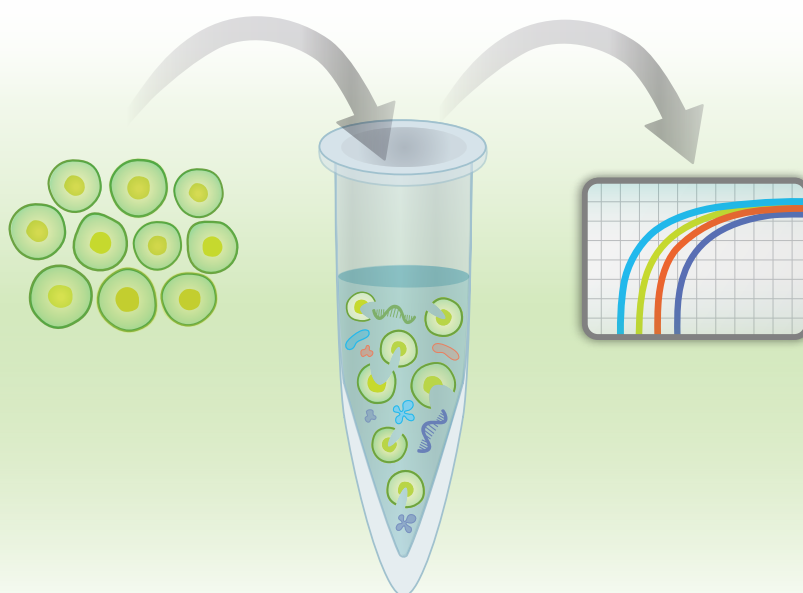


PCR Reagents: SingleShot™ Cell Lysis RT-qPCR Kits

Bulletin 6604

## What is SingleShot?

SingleShot is a family of kits that provide a complete and fast solution for generation of lysates from cell cultures. These lysates are optimized for downstream one- or two-step quantitative PCR (qPCR) reactions, and do not require an RNA purification step.



## Why use SingleShot Cell Lysis RT-qPCR Kits?

- As few as 10 to as many as 100,000 cells can be used
- Complete removal of genomic DNA (gDNA) without the need for purification
- Preservation of RNA integrity by an RNase inhibitor
- No loss of rare transcripts from column purification
- Optimal accuracy and high sensitivity of qPCR data
- Validated with PrimePCR™ Assays and Panels
- Simple, short protocol suitable for automation and high-throughput experiments
- Comparable results to those obtained when using purified RNA

Visit [bio-rad.com/web/SUITKits](http://bio-rad.com/web/SUITKits) for more information.

**BIO-RAD**

### How does SingleShot work?

Minimal hands-on protocol provides a high-throughput solution (Figure 1). Grow cells to desired density, transfer up to 10<sup>5</sup> cells to the wells of a PCR plate, add lysis solution, and incubate at room temperature for 10 min. Heat for 5 min at 37°C, then for 5 min at 75°C. The lysate is ready for reverse transcription qPCR (RT-qPCR) or can be stored for future use.

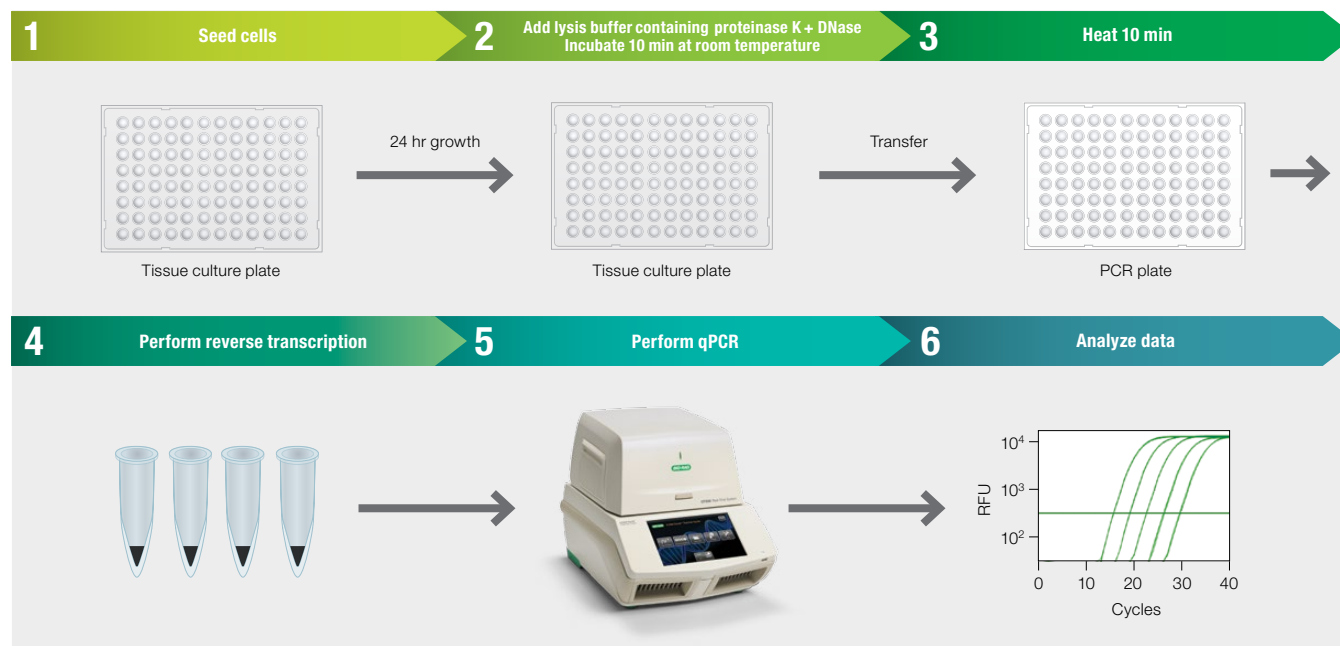
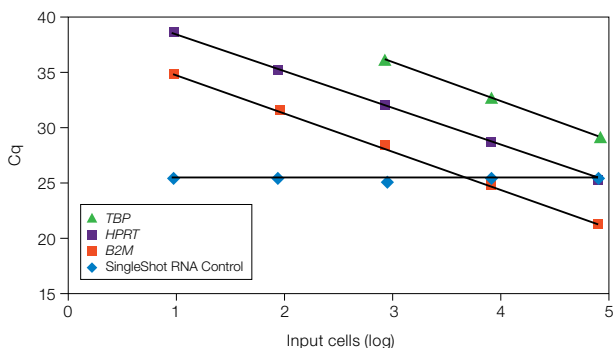


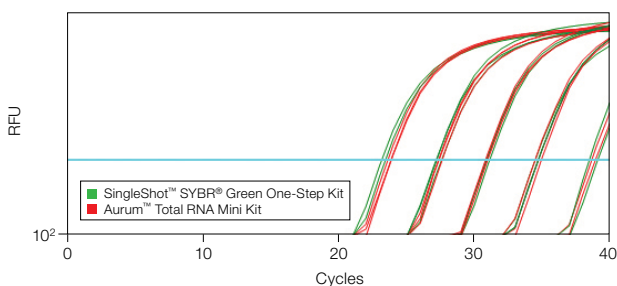
Fig. 1. SingleShot workflow.

### How do SingleShot Kits perform and compare to others?

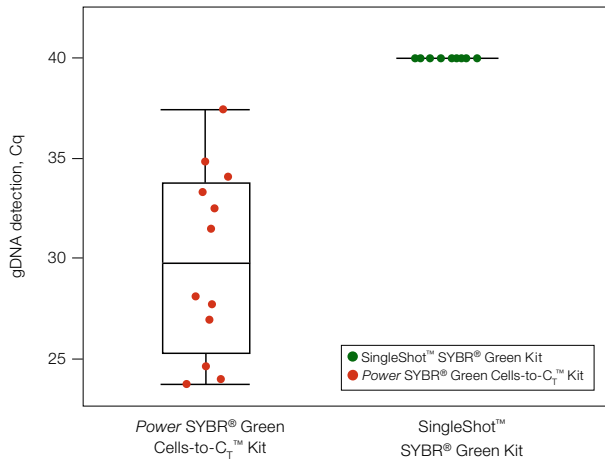
SingleShot Kits produce consistent results and superior performance compared to competitor kits (Figures 2–4).



**Fig. 2. SingleShot Probes Kit maintains high PCR efficiencies across all expression levels.** Accurate gene expression studies require that PCR efficiencies be within 90–110% per the minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines. Using the SingleShot Probes Kit, three targets of varying expression levels were analyzed using input cell numbers ranging from 10<sup>5</sup> to 10 HeLa cells. Greater than 98% PCR efficiencies were maintained regardless of the expression levels (*TBP* ▲, 99.6%; *HPRT* ■, 98.6%; *B2M* ■, 99.2%) and sensitivity down to 10 cells was observed. The SingleShot RNA Control (◆) was used to monitor PCR inhibition. As the control quantification cycle (Cq) values remained constant, no inhibition was noted.



**Fig. 3. SingleShot™ SYBR® Green One-Step Kit lysates yield a dynamic range similar to that of purified RNA.** Gene expression data generated using SingleShot™ SYBR® Green One-Step Kit lysates and isolated RNA (Aurum Total RNA Mini Kit) were compared using a human cell line (K652) and 10<sup>5</sup>–10 input cells. *B2M* expression was assessed using a PrimePCR qPCR Assay. SingleShot™ SYBR® Green One-Step Kit lysates showed a dynamic range similar to that of the RNA generated using the Aurum Total RNA Mini Kit without the need for RNA isolation. RFU, relative fluorescence units.



**Fig. 4. SingleShot™ SYBR® Green Kit efficiently removes gDNA from cell lysates.** Accurate gene expression studies are based on relative expression levels of RNA transcripts and require qPCR amplification of the respective cDNA copies without gDNA amplification. When qPCR assays are designed, best practice is to design them to avoid gDNA amplification. However, in cases where this is not possible, it is important to eliminate gDNA prior to cDNA synthesis. Utilizing the PrimePCR DNA Contamination Assay (designed to amplify only gDNA) and the SingleShot™ SYBR® Green Kit (both from Bio-Rad) and the Power SYBR® Cells-to-C<sub>T</sub>™ Kit (Life Technologies), 10<sup>4</sup>–10<sup>5</sup> input cells were evaluated for gDNA carryover after DNase treatment. The SingleShot Kit completely removed gDNA in the three different cell lines tested (HeLa, NCI-H1975, and NIH 3T3) with no amplification from the DNA control assay, regardless of the starting amount of input cells (●). The competitor kit amplified gDNA at high levels across all three cell lines at all starting amounts of input cells (●), which can cause inaccurate gene expression data because both cDNA and gDNA would be amplified. Cq, quantification cycle; NCI, National Cancer Institute; NIH, National Institutes of Health.

### What cell lines have been validated with SingleShot Kits?

SingleShot Cell Lysis RT-qPCR Kits have been validated for use with a number of common human and rodent cell lines, such as all types of adherent and suspension cells including, but not limited to, those listed in Table 1.

**Table 1. SingleShot Kits validated for use with common cell lines.**

Cell Line	Property	Species
A549	Adherent	<i>Homo sapiens</i> , lung carcinoma
HeLa	Adherent	<i>H. sapiens</i> , cervical adenocarcinoma
HepG2	Adherent	<i>H. sapiens</i> , liver carcinoma
MCF-7	Adherent	<i>H. sapiens</i> , epithelial, adenocarcinoma
NCI-H1975	Adherent	<i>H. sapiens</i> , lung adenocarcinoma
NIH 3T3	Adherent	<i>Mus musculus</i> , embryonic fibroblast
HEK 293	Suspension	<i>H. sapiens</i> , kidney
Jurkat	Suspension	<i>H. sapiens</i> , T lymphocyte
K562	Suspension	<i>H. sapiens</i> , chronic myelogenous leukemia
PC-12	Suspension	<i>Rattus norvegicus</i> , pheochromocytoma, adrenal
HepaRG	Primary-like	<i>H. sapiens</i> , hepatocyte

### Want more details?

Table 2 provides an overview of SingleShot and competitor kits.

**Table 2. Comparison of SingleShot and competitor kits.**

Property	Bio-Rad SingleShot Kits	Life Technologies TaqMan Cells-to-C <sub>T</sub> Kits	QIAGEN FastLane Cell Kits
gDNA clearance	***	*	**
RNA preservation	***	**	**
Input cells	10–100,000	10–100,000	Up to 10,000
RNA control with kit	***	Sold separately	—
Two-step kits	***	***	Lysis kit only
One-step kits	***	—	***
Automation friendly	***	**	**

\* Fair  
 \*\* Good  
 \*\*\* Excellent  
 — Not applicable

### What is included in each kit and how do I order them?

The brief descriptions and ordering information in Table 3 will help you select an appropriate SingleShot Kit.

**Table 3. Ordering information.**

Kit Type	Kit Description	Catalog Number	Kit Contents
SingleShot Two-Step RT-qPCR Kits	SingleShot™ SYBR® Green Kit, 100 x 50 µl reactions	172-5085	<ul style="list-style-type: none"> <li>■ SingleShot Cell Lysis Reagents</li> <li>■ iScript™ Advanced cDNA Synthesis Kit for RT-qPCR (#172-5038)</li> <li>■ SsoAdvanced™ Universal SYBR® Green Supermix (#172-5271)</li> <li>■ SingleShot RNA Control (enables the determination of optimal input cells and lysates)</li> </ul>
	SingleShot Probes Kit, 100 x 50 µl reactions	172-5090	<ul style="list-style-type: none"> <li>■ SingleShot Cell Lysis Reagents</li> <li>■ iScript Advanced cDNA Synthesis Kit for RT-qPCR (#172-5038)</li> <li>■ SsoAdvanced Universal Probes Supermix (#172-5281)</li> <li>■ SingleShot RNA Control (enables the determination of optimal input cells and lysates)</li> </ul>
SingleShot One-Step RT-qPCR Kits	SingleShot™ SYBR® Green One-Step Kit, 100 x 50 µl reactions	172-5095	<ul style="list-style-type: none"> <li>■ SingleShot Cell Lysis Reagents</li> <li>■ iTaq™ Universal SYBR® Green One-Step Kit (#172-5150)</li> <li>■ SingleShot RNA Control (enables the determination of optimal input cells and lysates)</li> </ul>
	SingleShot Probes One-Step Kit, 100 x 50 µl reactions	172-5070	<ul style="list-style-type: none"> <li>■ SingleShot Cell Lysis Reagents</li> <li>■ iTaq Universal Probes One-Step Kit (#172-5140)</li> <li>■ SingleShot RNA Control (enables the determination of optimal input cells and lysates)</li> </ul>
SingleShot Cell Lysis Kits*	SingleShot Cell Lysis Kit, 100 x 50 µl reactions	172-5080	<ul style="list-style-type: none"> <li>■ SingleShot Cell Lysis Reagents</li> <li>■ <b>RT-qPCR reagents sold separately:</b> <ul style="list-style-type: none"> <li>– Reverse transcription: iScript Advanced cDNA Synthesis Kit for RT-qPCR (#172-5038)</li> <li>– 2-step RT-qPCR: SsoAdvanced™ Universal SYBR® Green Supermix (#172-5271); SsoAdvanced Universal Probes Supermix (#172-5281)</li> <li>– 1-step RT-qPCR: iTaq™ Universal SYBR® Green One-Step Kit (#172-5150); iTaq Universal Probes One-Step Kit (#172-5140)</li> </ul> </li> </ul>
	SingleShot Cell Lysis Kit, 500 x 50 µl reactions	172-5081	Kit contents same as #172-5080

\* Required RT-qPCR reagents are sold separately.

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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 Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.

The use of iTaq and SsoAdvanced Supermixes is covered by one or more of the following U.S. patents and corresponding patent claims outside the U.S.: 5,804,375; 5,538,848; 5,723,591; 5,876,930; 5,994,056; 6,030,787; 6,171,785; and 6,258,569. The purchase of these products includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, are conveyed expressly, by implication, or by estoppel. These products are for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.



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