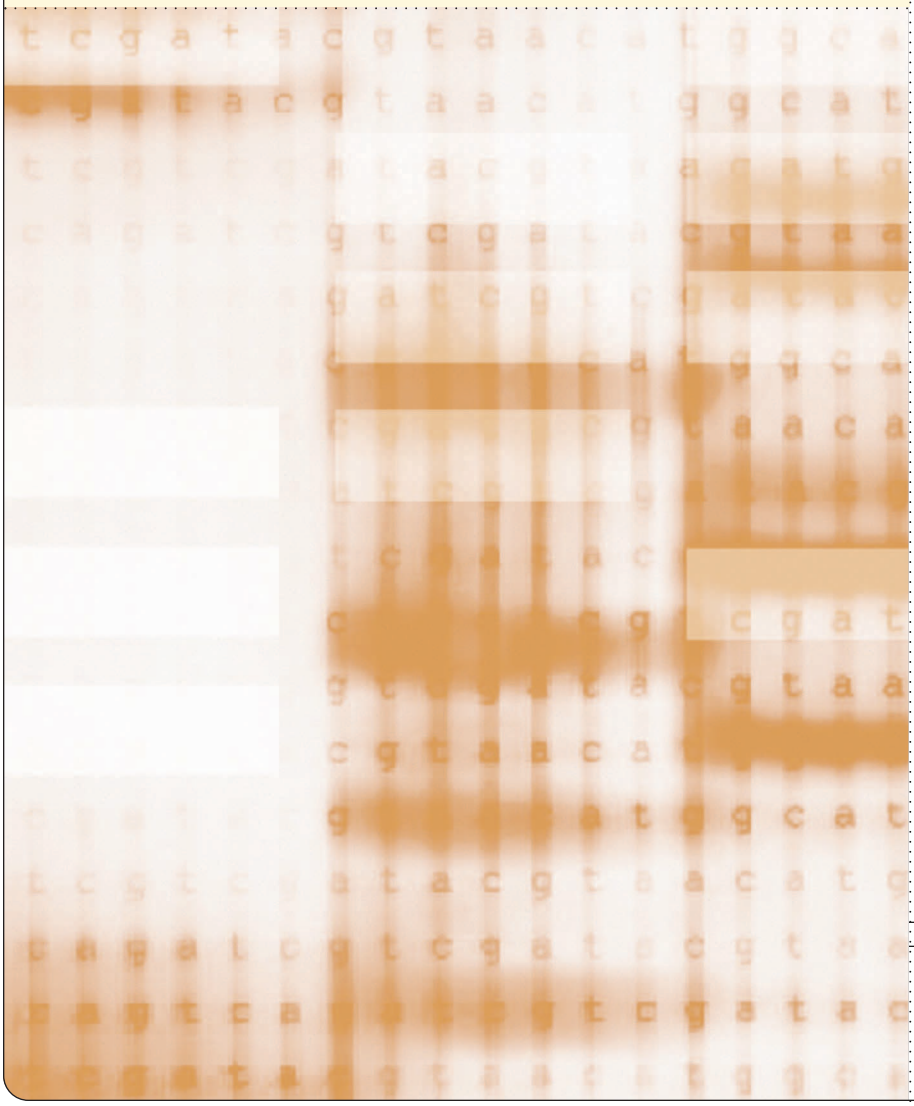
SideStep™ II Lysis and Stabilization Products

- Immediate cell lysis and RNA stabilization for at least 20 months at -80°C
- Novel, single-copy Quantos™ QPCR normalization primers included
- Samples are ready for QRT-PCR in 10 minutes
- Easy DNase treatment protocol allows use of your existing primers
- No toxic organic extractions or precipitation steps

OUR SIDESTEP™ II QRT-PCR PRODUCTS ALLOW YOU TO QUANTITATE GENE EXPRESSION DIRECTLY FROM CELLS. THESE KITS DELIVER EFFICIENT CELL LYSIS, RNA STABILIZATION, gDNA REMOVAL, AND QUANTITATIVE GENE EXPRESSION ANALYSIS—WITHOUT RNA PURIFICATION. SIDESTEP II QRT-PCR PRODUCTS ARE IDEAL FOR SCREENING MULTIPLE SAMPLES FIRST SO YOU NEED ONLY PURIFY THE MOST RELEVANT, INTERESTING SAMPLES.



Our next generation SideStep™ II quantitative reverse transcription PCR (QRT-PCR) products allow you to skip RNA purification and go directly to QRT-PCR (Figures 1 and 2). Only our patent pending technology stabilizes RNA in a cell lysate for at least 20 months at -80°C using a single tube format, minimizing RNA loss and degradation. We also include three Quantos™ QPCR Normalization Primer Sets, each of which amplifies a unique single-copy human DNA sequence for the precise quantitation of gDNA. You can accurately determine cell number and use this value for ΔΔCt gene expression calculations (Figure 3). Additionally, our kits include reagents that remove genomic DNA from SideStep cell lysates giving you flexibility in your primer design by eliminating the need to use primers that span exon junctions.

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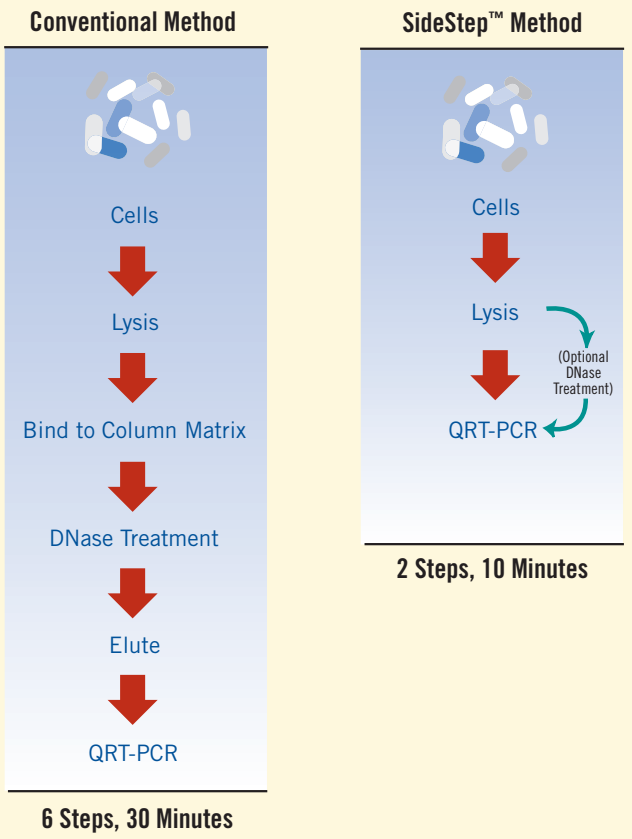


Figure 1
SideStep™ Products Save Time, Increase Throughput
Our SideStep™ product line takes you directly from cells to QRT-PCR in fewer steps with less time, increasing your throughput.

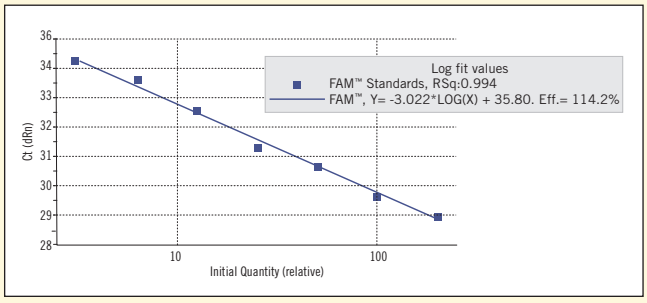
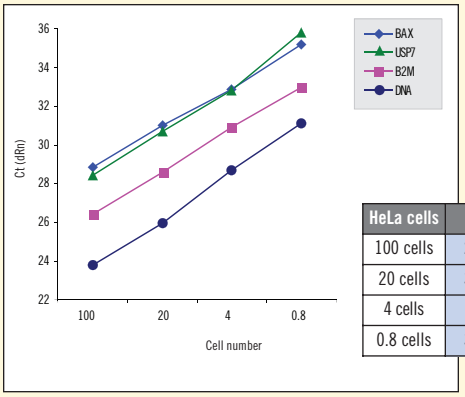


Figure 2
Gene Expression Directly from HeLa Cells
We generated a standard curve using 2-fold dilutions of SideStep™ cell lysate with the SideStep™ II QRT-PCR Master Mix, 1-step and USP-7 TaqMan® primers and probe.

A



HeLa cells	BAX	USP7	B2M	DNA
100 cells	28.87	38.41	26.44	23.79
20 cells	31.00	30.72	28.61	25.95
4 cells	32.84	32.79	30.92	28.70
0.8 cells	35.17	35.77	32.95	31.10

Sample #1 - 100 HeLa cells; Sample #2 - 20 HeLa cells

B

	100 cells	20 cells	
BAX (GOI)	28.87	31.00	ΔΔCt.
DNA (Norm)	23.79	25.95	
B2M (Norm)	26.44	28.61	
Δ Ct (GOI-Norm-DNA)	5.08	5.05	0.03
Δ Ct (GOI-Norm-B2M)	2.43	2.39	0.04

Fold change = $2^{\Delta\Delta Ct} = 2^{0.03} = 1.021$ (Norm-DNA)
Fold change = $2^{\Delta\Delta Ct} = 2^{0.04} = 1.028$ (Norm-B2M)

Figure 3
Equivalent Comparative Quantification of DNA using SideStep™ Cell Lysates
Panel A — QPCR (SYBR® Green) and QRT-PCR (TaqMan®) with SideStep™ HeLa Cells Lysate
We performed QRT-PCR using our Brilliant® QRT-PCR Master Mix, 1-Step with BAX, USP7, and B2M TaqMan® primers and probe (ABI) with a 5-fold serially diluted SideStep™ II cell lysate on the Mx3000P® QPCR System. QPCR was carried out using the SideStep™ II SYBR® Green QPCR Master Mix, Quantos™ QPCR Normalization Primer on the Mx3000P® QPCR System. The data show good correlation between DNA and RNA amounts dependent on the number of cells per reaction.

Panel B — BAX Gene Expression in HeLa Cells Using B2M or DNA as the Normalizer
The fold difference in BAX gene expression using a housekeeping gene (B2M) vs. genomic DNA is equivalent (1.028 vs 1.021). These results demonstrate that QPCR amplification of a unique single-copy gDNA sequence is an effective tool to calculate relative quantities of specific mRNA transcripts in multiple samples.

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SideStep™ Lysis and Stabilization Products:

SideStep™ II Cell Lysis Analysis Kit	100 lysis rxn	400916
SideStep™ II QRT-PCR Master Mix, 1-Step	400 rxn	400917
SideStep™ II QRT-PCR Master Mix, 2- Step	400 rxn	400918
SideStep™ II SYBR® Green QRT-PCR Master Mix, 2-Step	400 rxn	400909
SideStep™ SYBR® Green QPCR Master Mix	400 rxn	400904
SideStep™ II QPCR cDNA Synthesis Kit	50 rxn	400908

a. Patents pending

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