

Nucleic Acid Stabilization in Cultured Cell and Tissue Lysates for QPCR Gene Expression Analysis

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SideStep™

*Gets Around
Nucleic Acid
Purification to
Real Time Gene
Expression
Analysis*

What is SideStep?

- **Easy to use lysis buffer for tissue and cultured cells**
- **One step protocol, lyses cells and stabilizes nucleic acids**
- **RNA Template for Stratagene's 1st strand cDNA synthesis kit**
- **RNA Template for one step QRT-PCR with probes**
- **DNA Template for QPCR with SYBR green or probes**

Features of SideStep

- **Lysis reaction volume is scalable**
- **Gives a QPCR signal equal to isolated RNA**
- **Use with Stratagene's Absolutely mRNA kit for mRNA from cells**
- **Compatible with Stratagene's Absolutely Total RNA Isolation Kits**

Simple Lysis Protocol for Cultured Cells

- Harvest and count cultured cells
- Resuspend cells in PBS
- Pellet 1,000 to 1,000,000 cells/ 1.5ml tube
- Vortex cells with 100ul SideStep buffer for 1 minute
- Nucleic acids stable for 6 months at -20°C

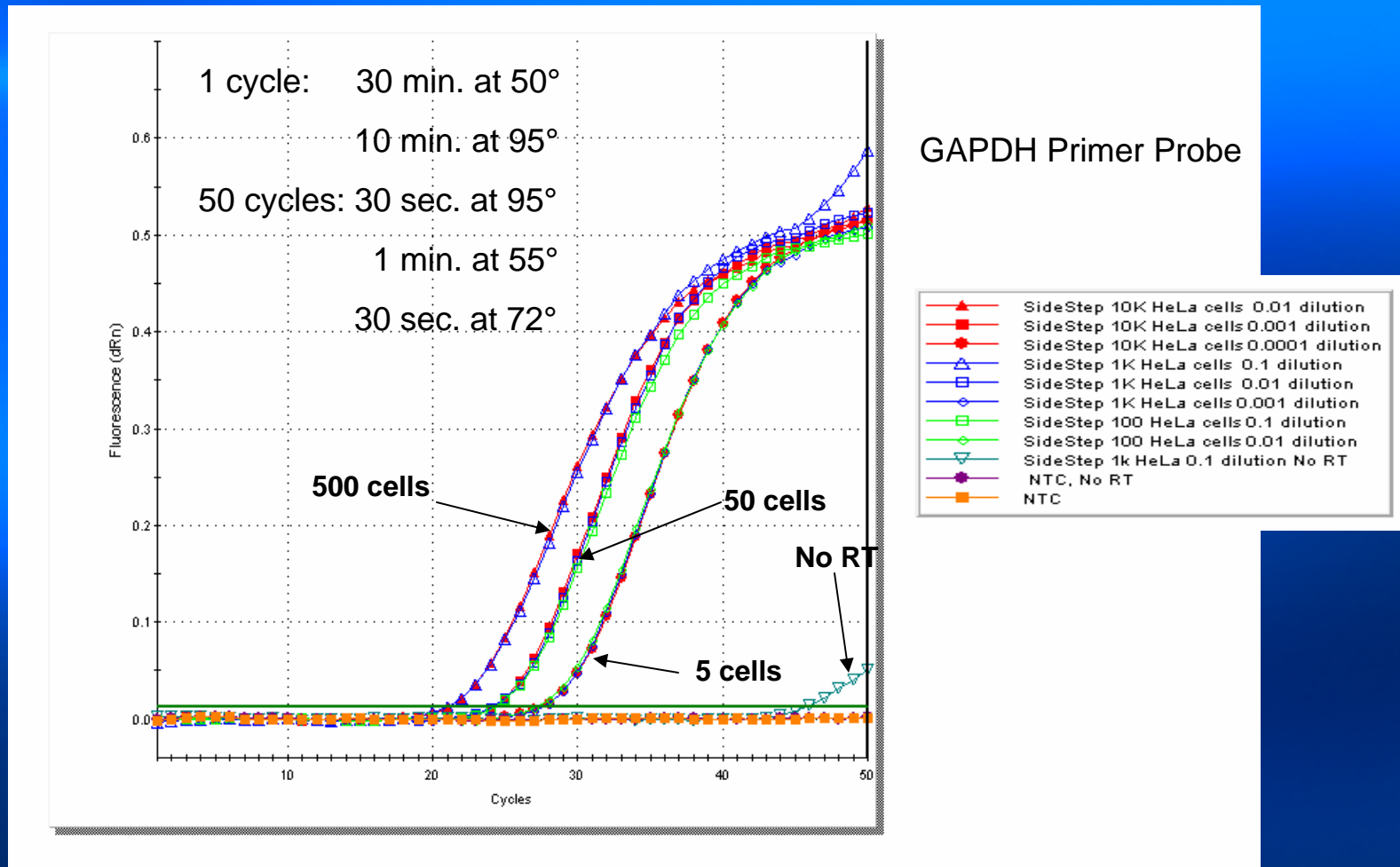
SideStep Lysate as Template for 1Step QRT-PCR

- Stratagene's Brilliant QRT-PCR Master mix for probes
- GAPDH primer probe sets specific for RNA
- QRT-PCR using the Mx 3000P

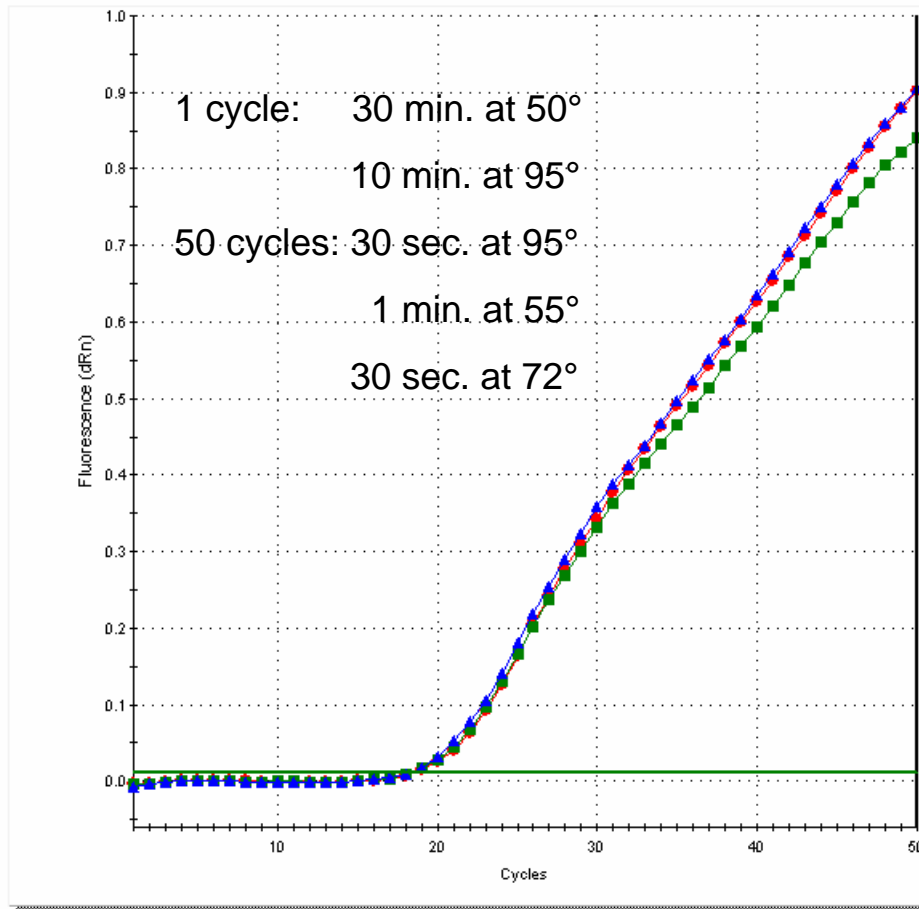


Brilliant One Step QRT-PCR for Probes

Regardless of initial lysate concentration, equal cell numbers give equal Ct's



Signal from SideStep is Equal to Purified RNA



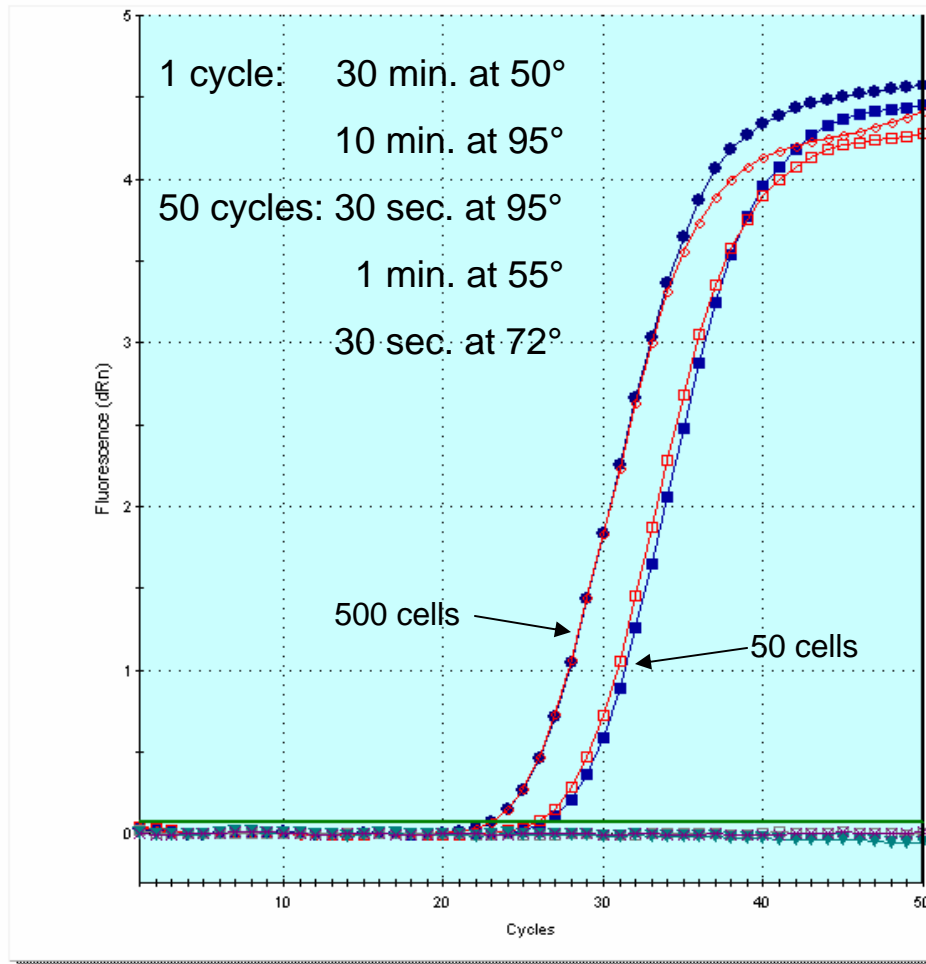
GAPDH Primer Probe

- 500 HeLa cells Absolutely RNA
- 500 HeLa cells SideStep
- 500 HeLa cells SideStep/Absolutely RNA

Nucleic Acid Stability

- **QRT-PCR of SideStep Lysate after 6 months of storage at -20°C**
- **BioAnalyzer 2100 Data showing RNA isolated from SideStep Lysate after 6 months of storage at -20°C.**
- **BioAnalyzer 2100 Data showing RNA isolated from SideStep Lysate after 8 hours at room temperature**

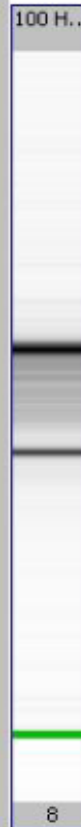
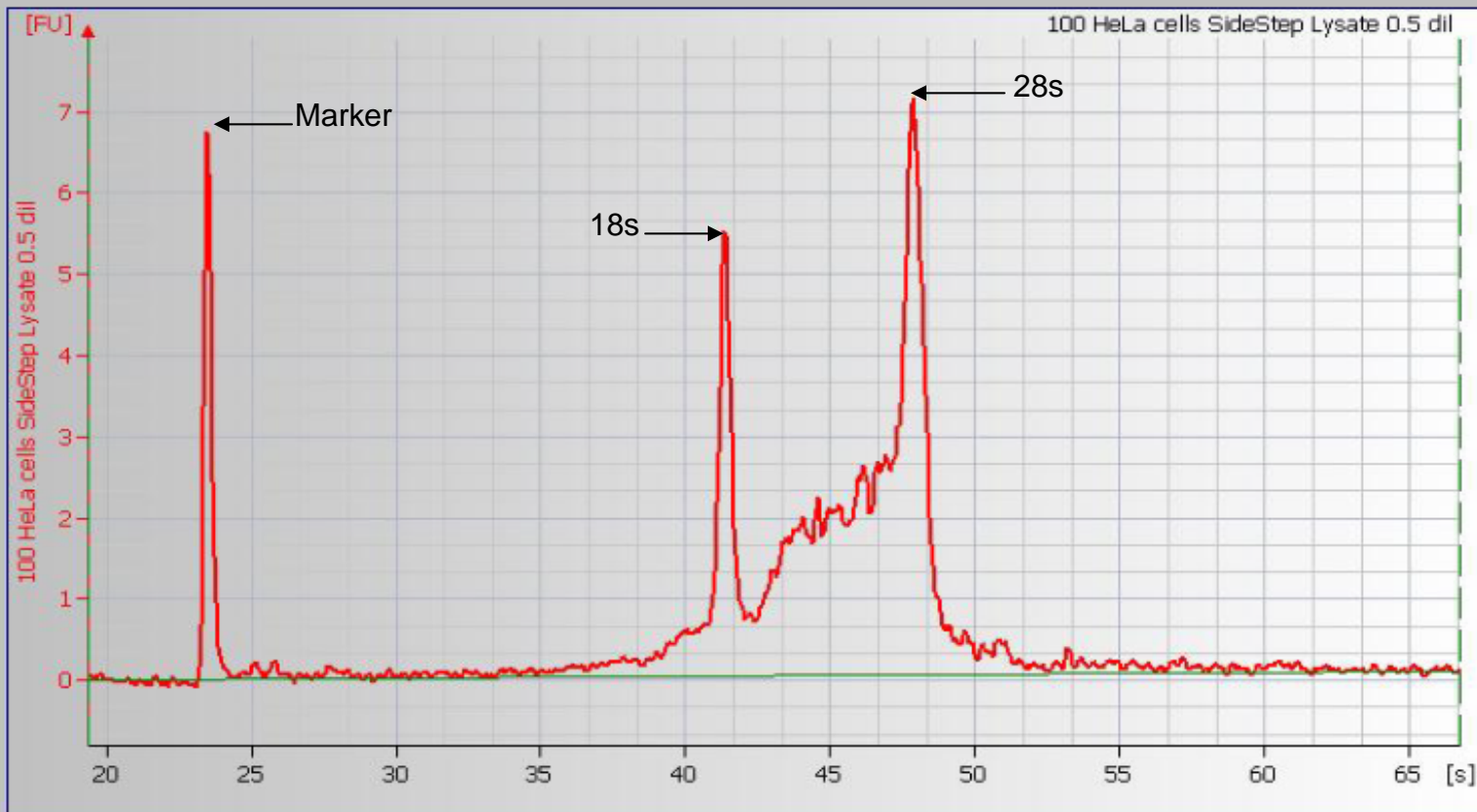
QRT-PCR of Column Purified RNA Stored at -80° C and SideStep Lysate Stored at -20° C After 6 Months



GAPDH Primer Probe

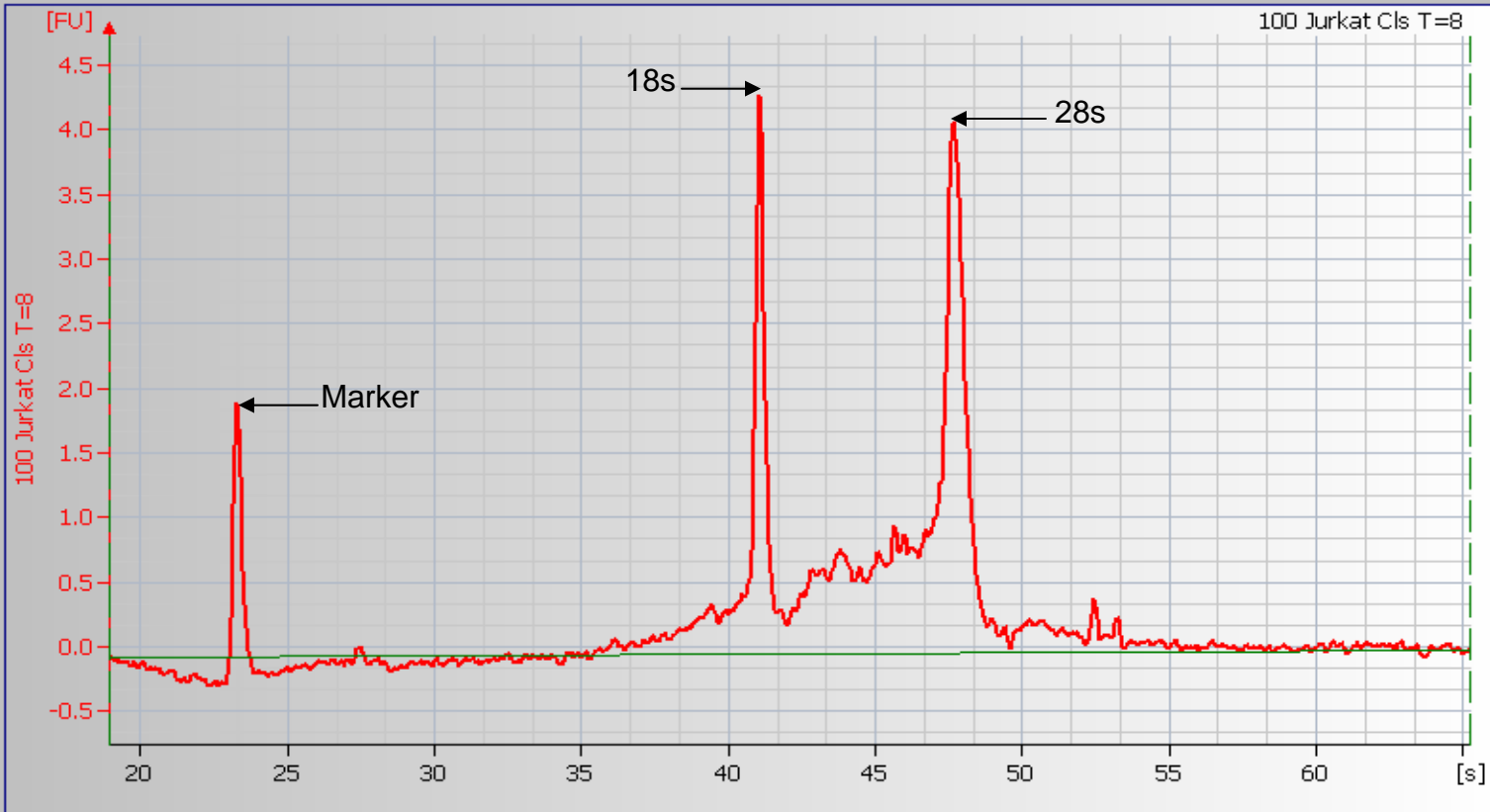
- HeLa RNA from 500 cells stored 6 mo. at -80 C
- HeLa RNA from 50 cells stored 6 mo. at -80 C
- ◇ HeLa SideStep Lysate 6 mo. old 500 cells
- HeLa SideStep Lysate 6 mo. old 50 cells
- No Template Control
- ▼ No RT with HeLA RNA
- × No RT with HeLA SideStep Lysate

Agilent 2100 Bioanalyzer Capillary Electrophoresis of RNA in SideStep Lysate of HeLa Cells Stored 6 Months at -20°C



RNA Area: 49.6
RNA Concentration: 953 pg/ μ l
rRNA Ratio [28s / 18s]: 1.9
RNA Integrity Number (RIN): 8.5

Agilent 2100 Bioanalyzer Capillary Electrophoresis of RNA in SideStep Lysate of Jurkat Cells After 8 Hours at Room Temperature

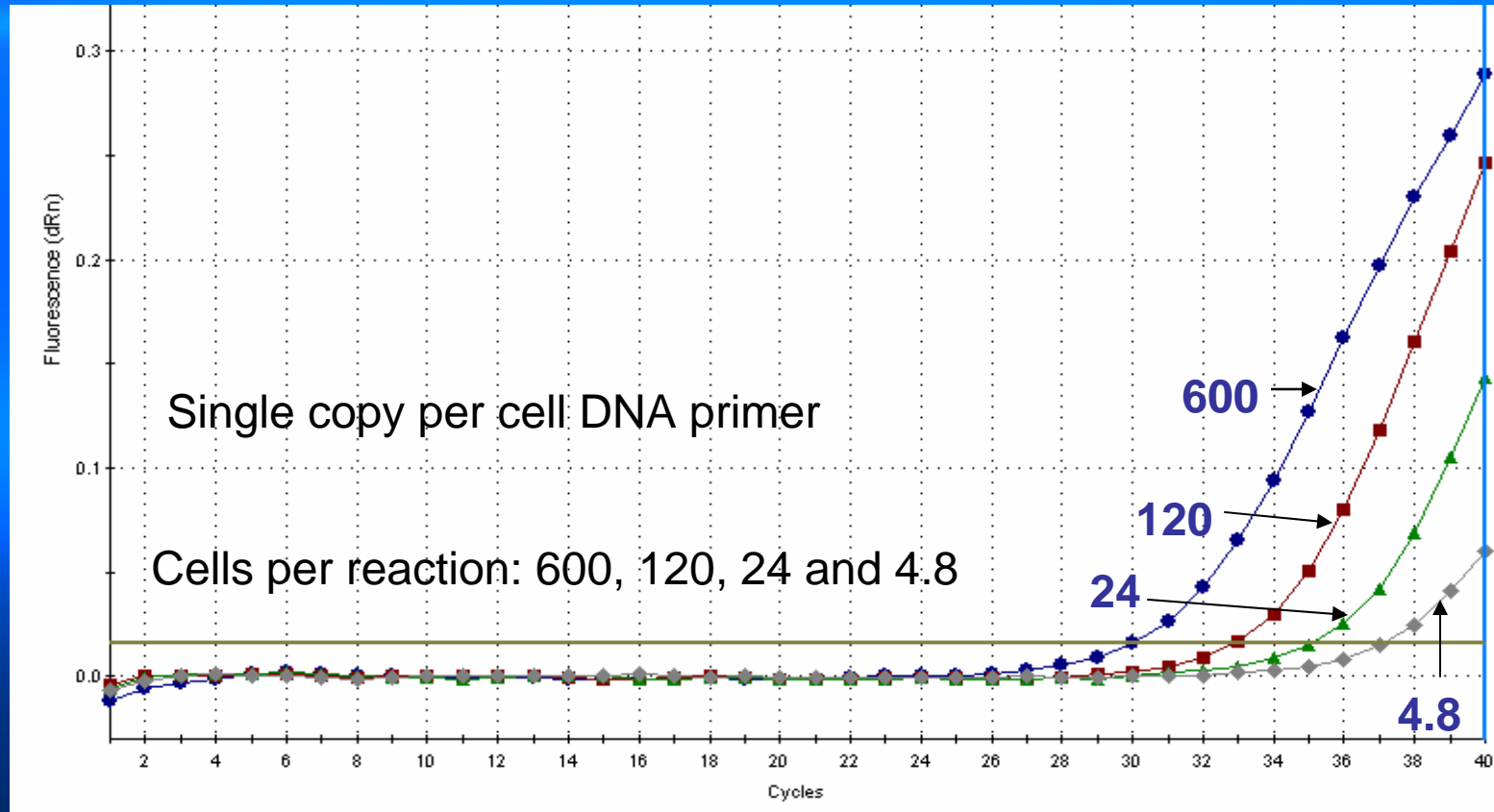


RNA Area: 24.0
RNA Concentration: 613 pg/ μ l
rRNA Ratio [28s / 18s]: 2.0
RNA Integrity Number (RIN): 7.9

QPCR with SYBR Green

- QPCR with single copy / cell DNA Primer set
- Use DNA to quantify input cell mass and normalize expression

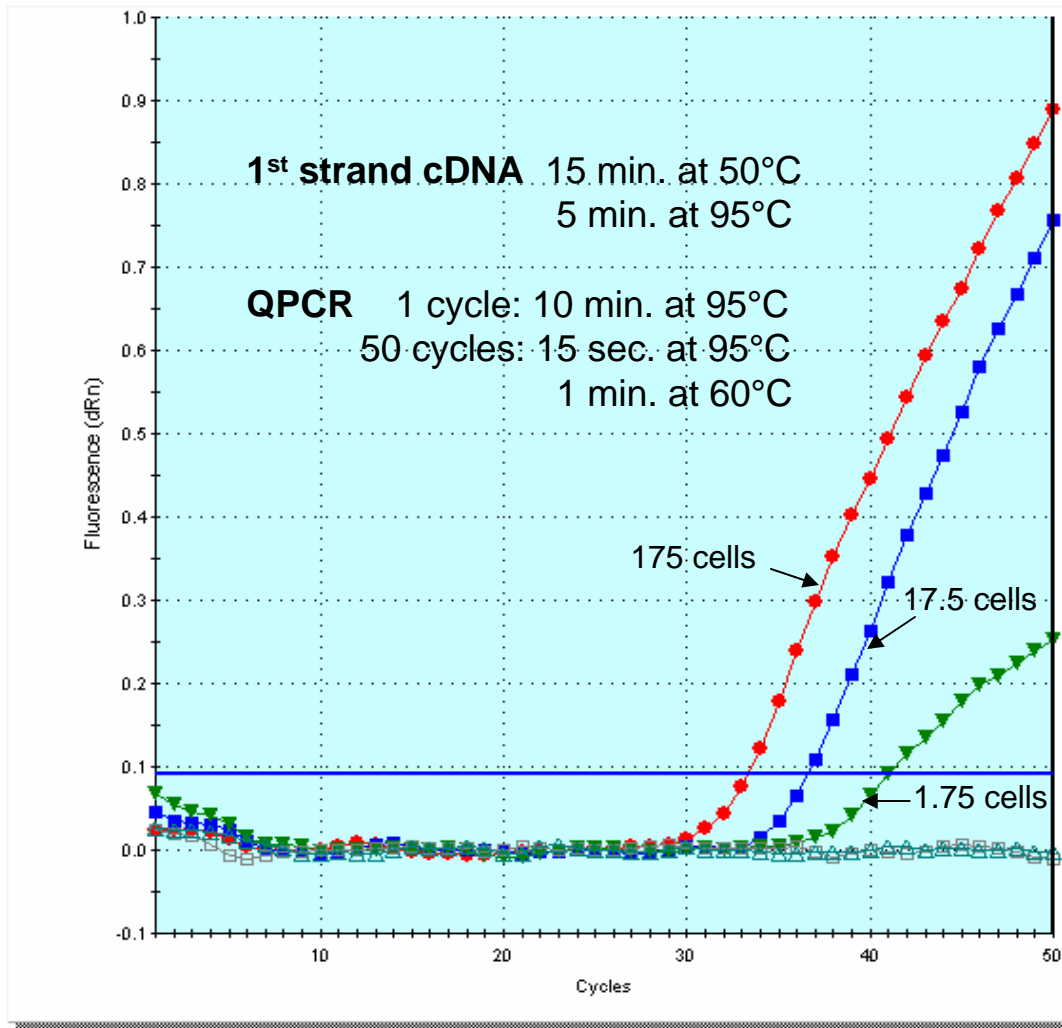
SideStep Lysate in Brilliant SYBR Green QPCR



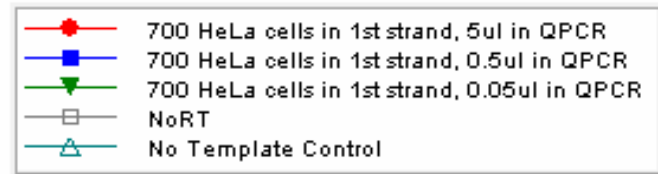
SideStep in 1st Strand cDNA Synthesis

- Add undiluted lysate directly to RT reactions
- Use SideStep up to 35% of RT reaction volume
- Use primer probe sets specific for RNA targets

SideStep Low Abundance Gene Expression



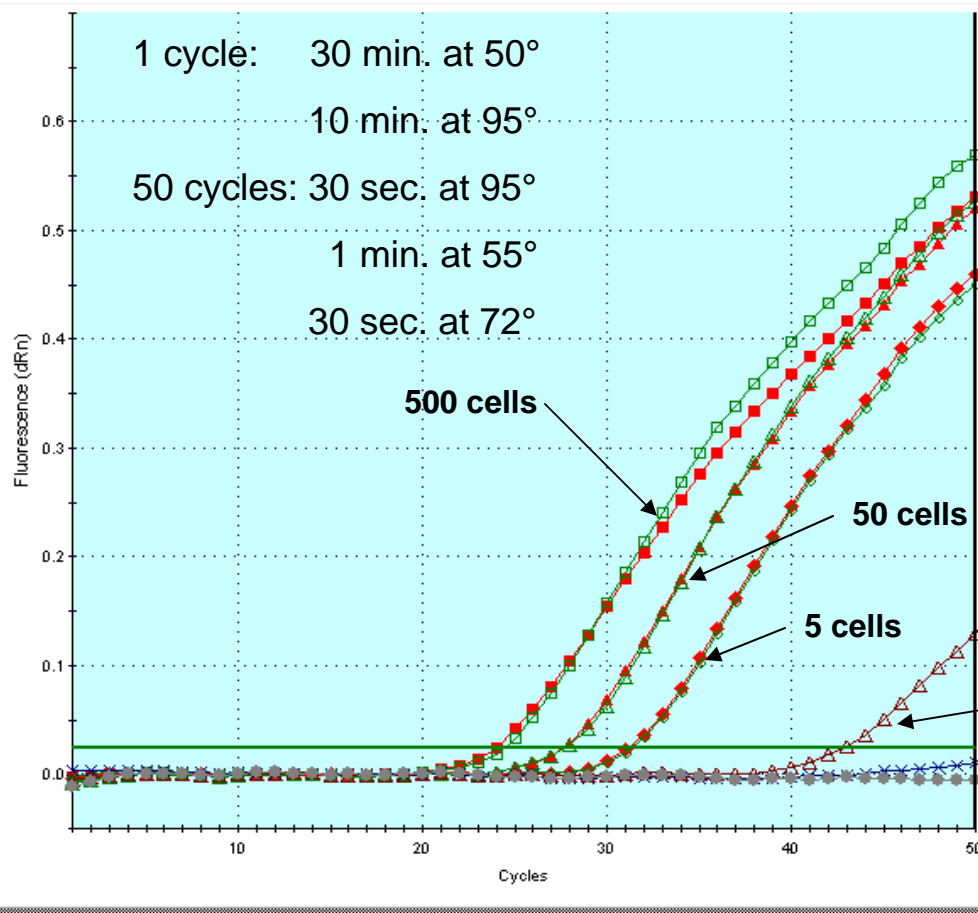
BAX Primer Probe



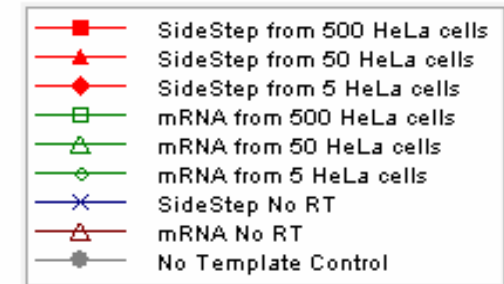
Absolutely mRNA isolation from SideStep lysate

- Simple addition to Absolutely mRNA isolation protocol
- Allows for Magnetic mRNA isolation from cells
- Signal from SideStep lysate equal to isolated mRNA

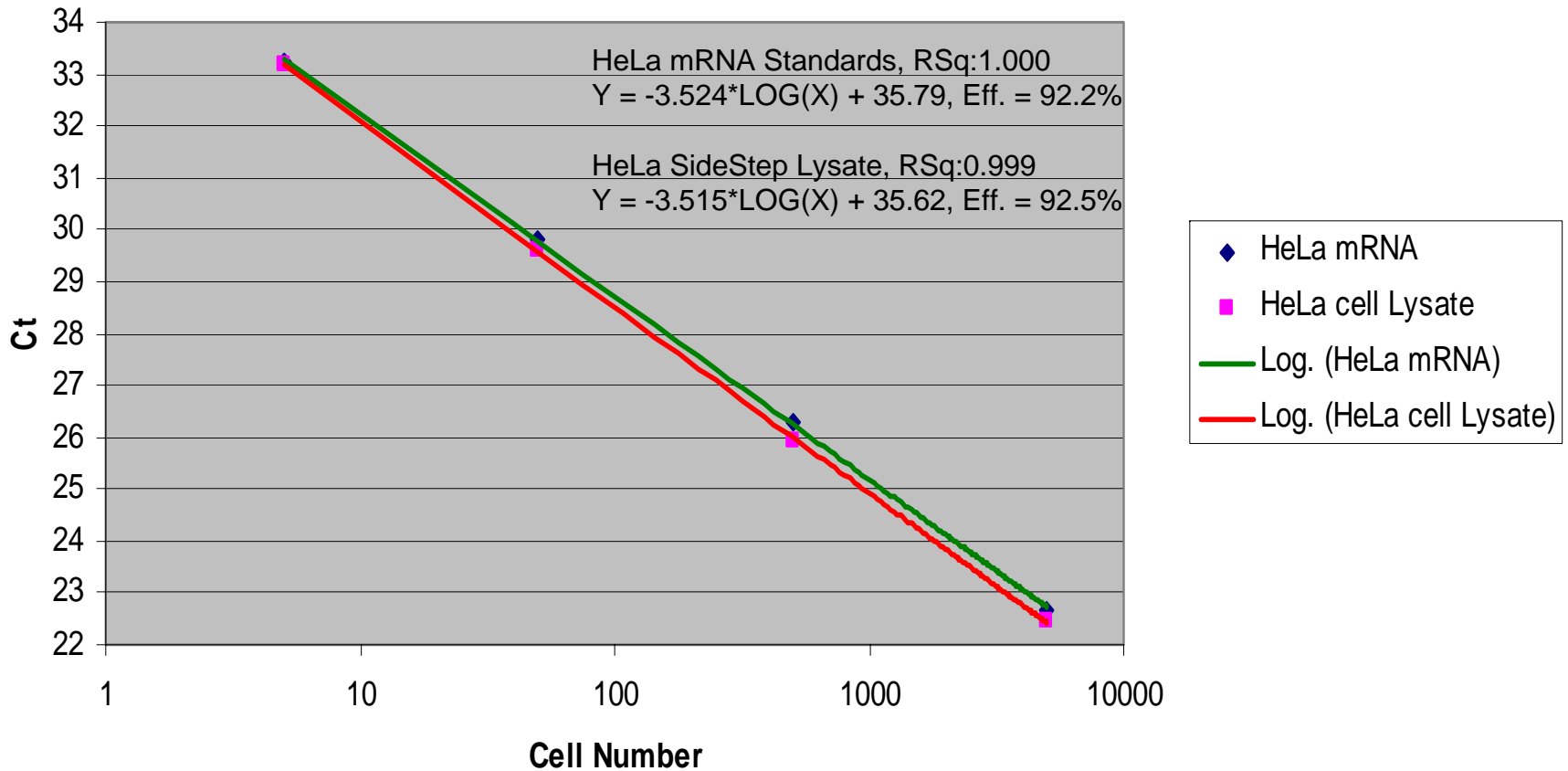
SideStep vs. Isolated mRNA



GAPDH Primer Probe



Standard Curves of HeLa mRNA Isolated from SideStep and HeLa SideStep Lysate

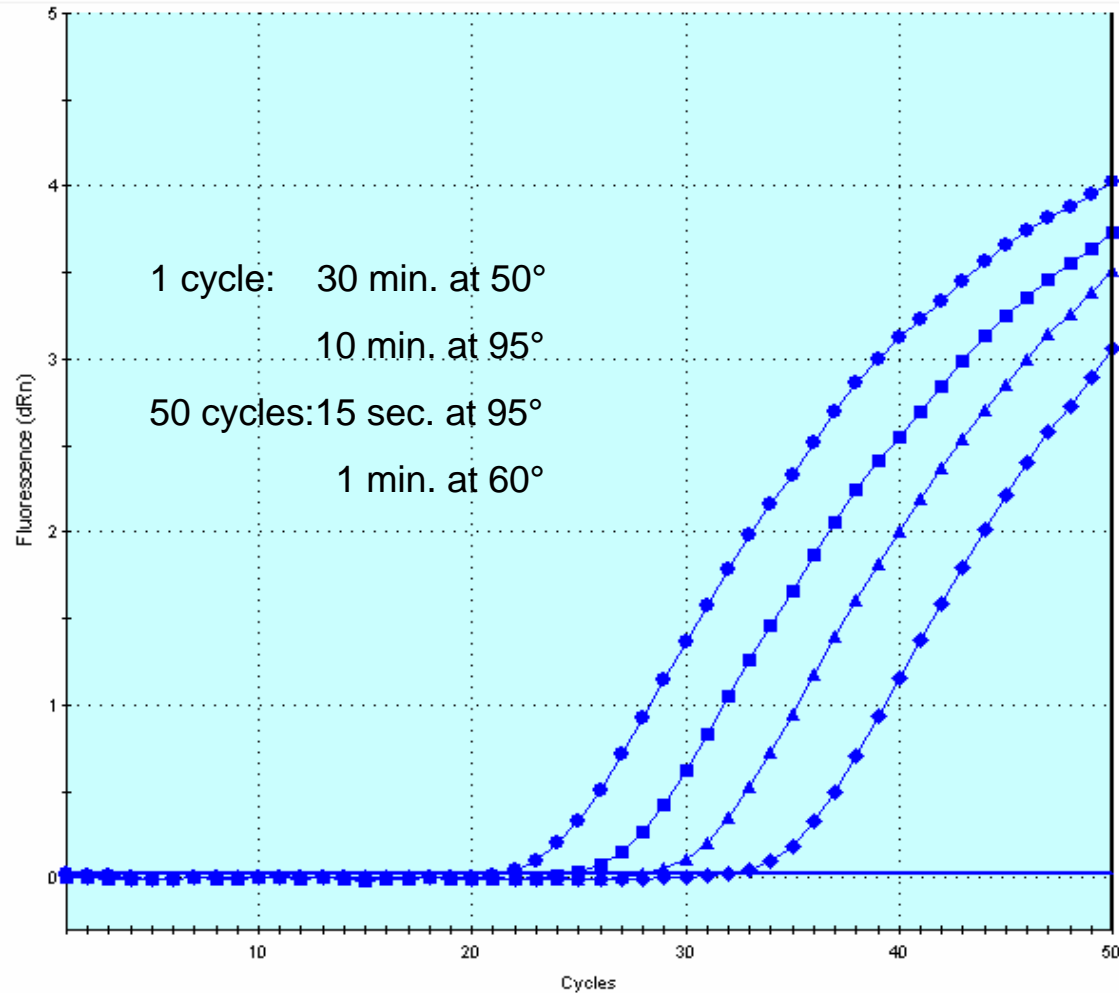


SideStep Tissue lysate Preparation

- Homogenize up to 20 mg of fresh or frozen tissue in 100ul of SideStep buffer.



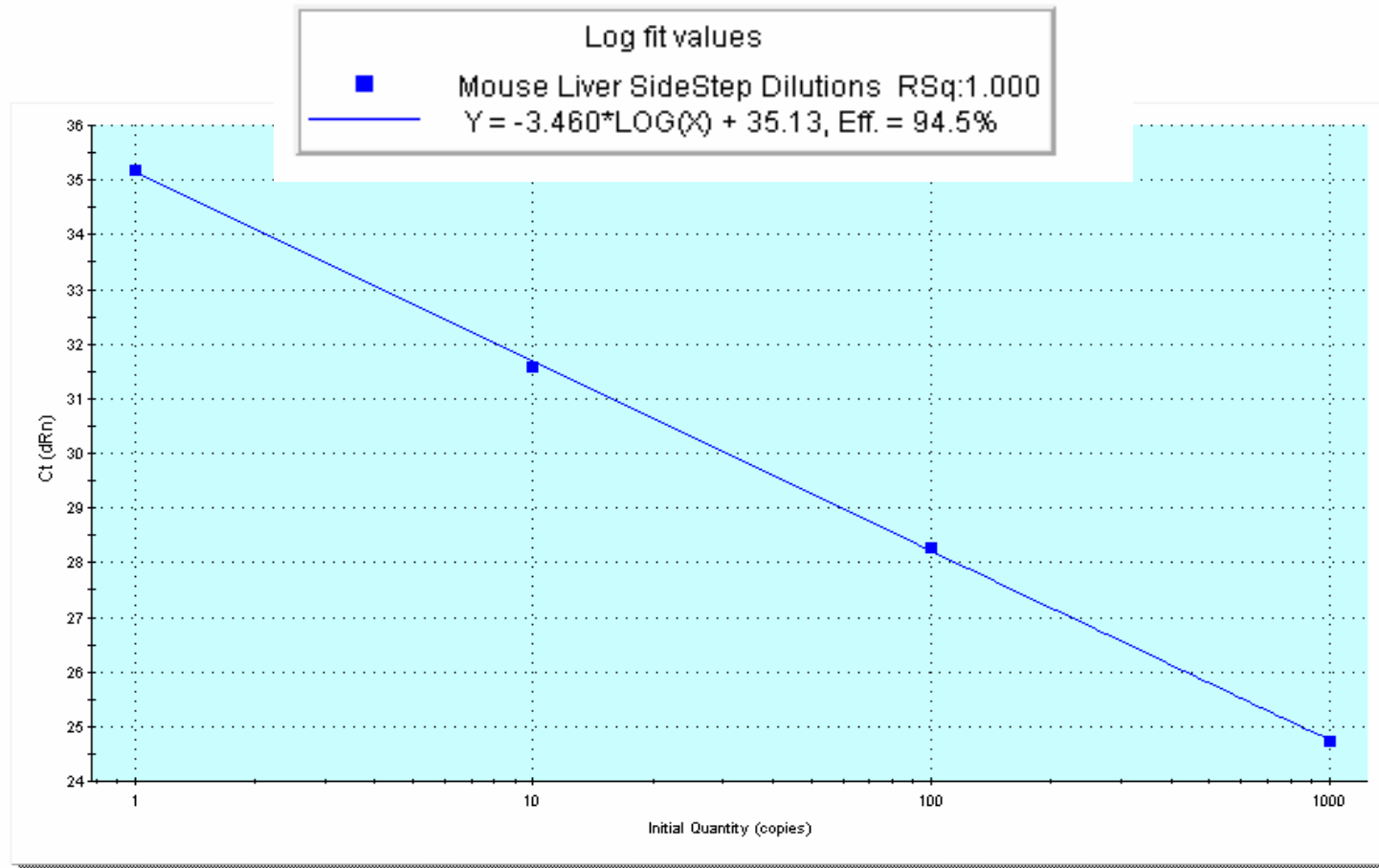
GAPDH Expression in SideStep Mouse Liver Lysate



GAPDH Primer Probe

- mouse liver 0.1 dilution
- mouse liver 0.01 dilution
- ▲ mouse liver 0.001 dilution
- ◆ mouse liver 0.0001 dilution

Standard Curve for Mouse Liver in SideStep



Conclusion

- **Simple protocol lyses cells and stabilizes nucleic acids**
- **SideStep lysate can be stored safely at -20°C for 6 months**
- **SideStep lysate makes RNA and DNA available for amplification**
- **Total RNA and mRNA can be isolated from SideStep lysate**