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

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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

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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

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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

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SideStep Lysate as Template for 1Step QRT-PCR

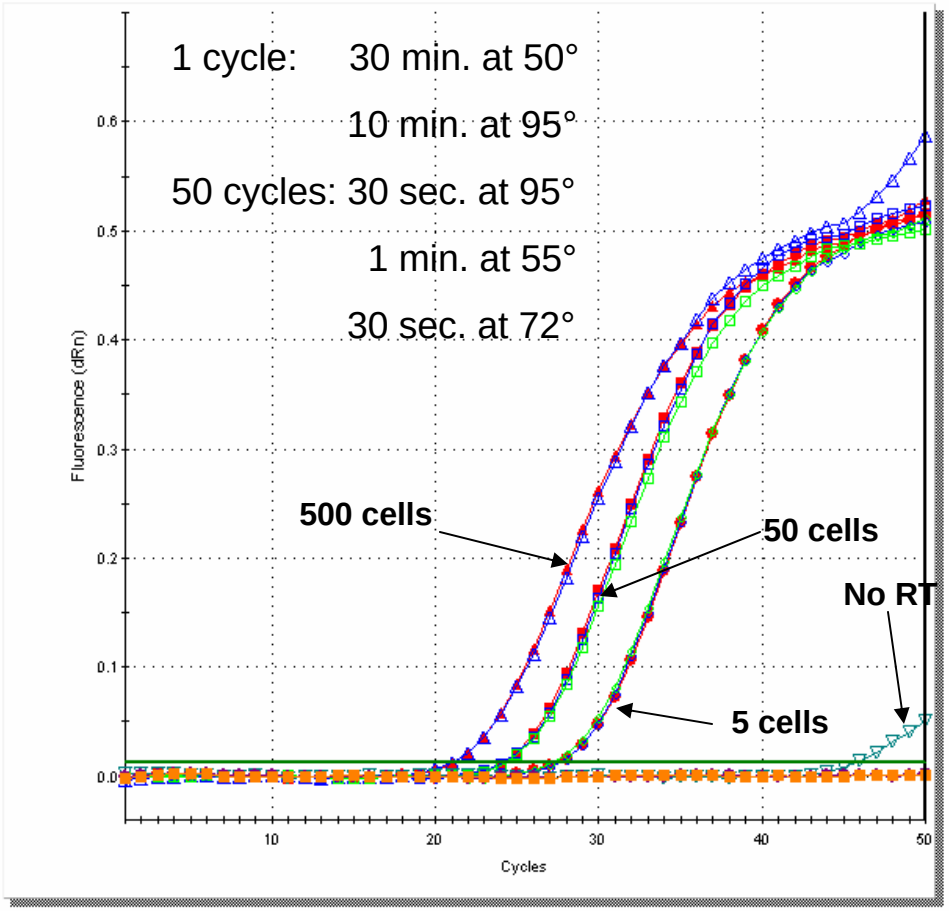
- Stratagene's Brilliant QRT-PCR Master mix for probes
- GAPDH primer probe sets specific for RNA
- QRT-PCR using the Mx 3000P



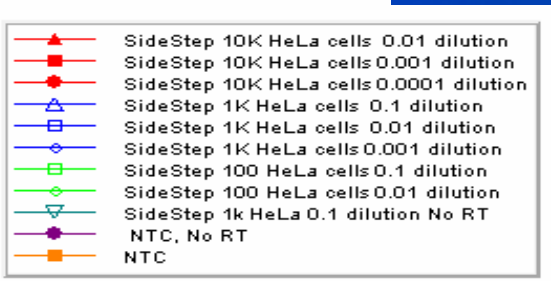
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Brilliant One Step QRT-PCR for Probes

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

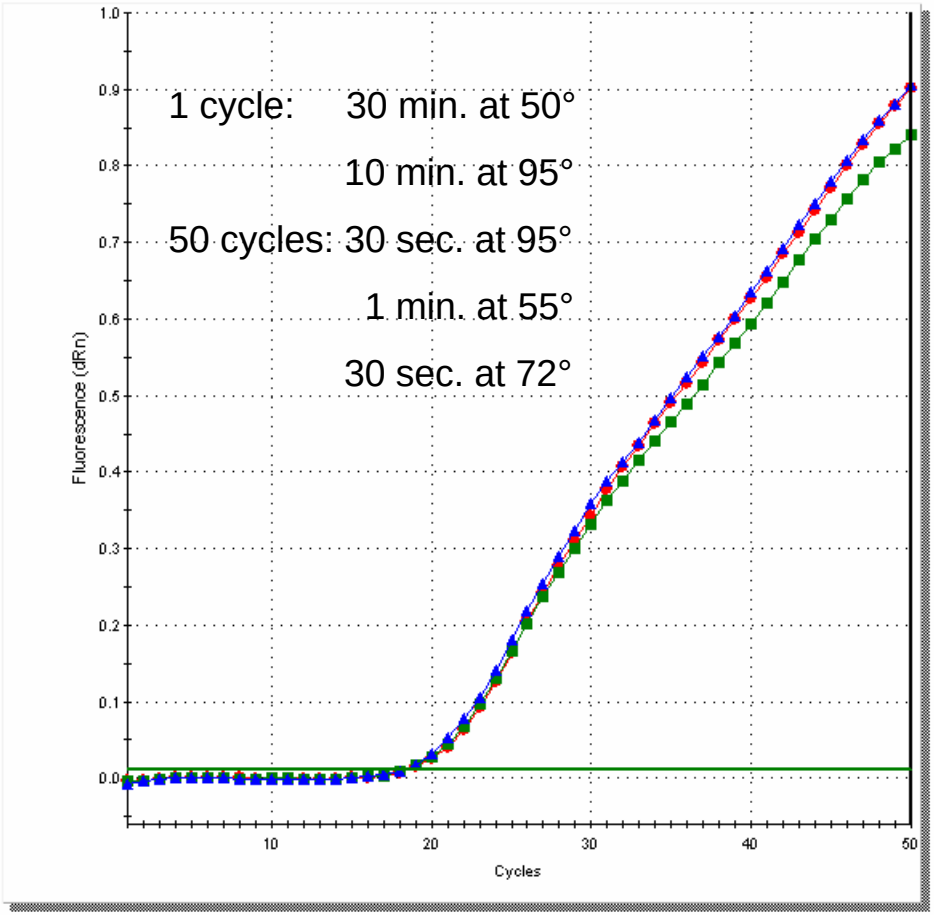


GAPDH Primer Probe



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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

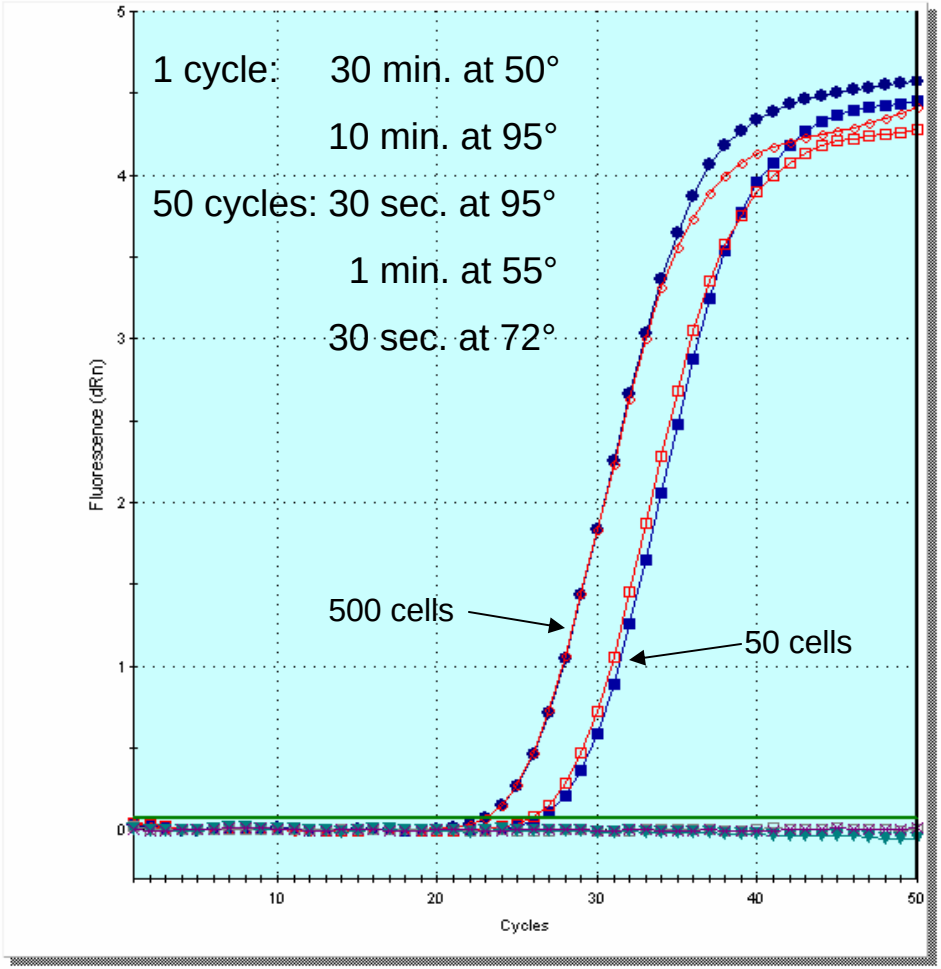
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Nucleic Acid Stability

- 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

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

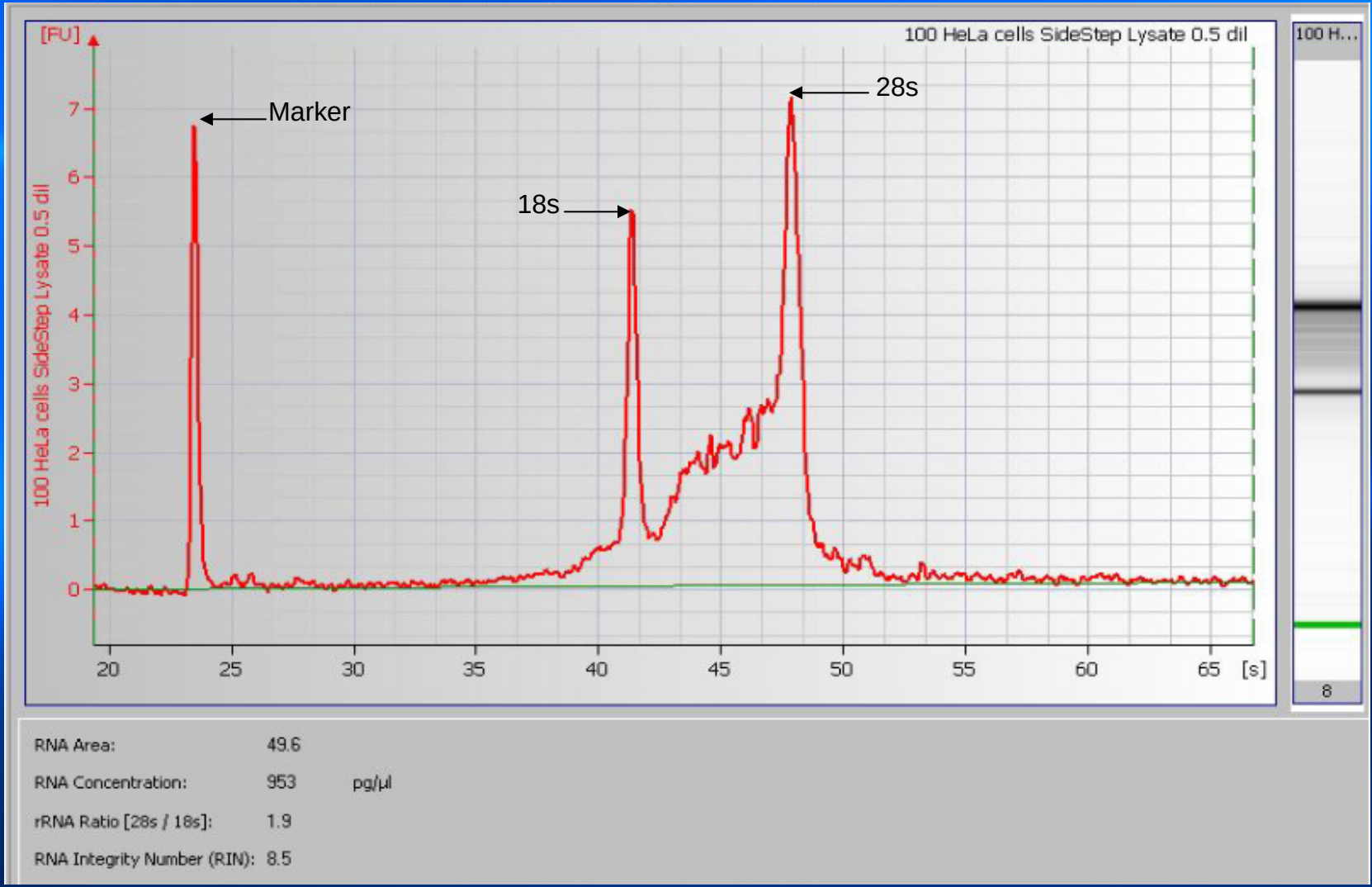


GAPDH Primer Probe

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

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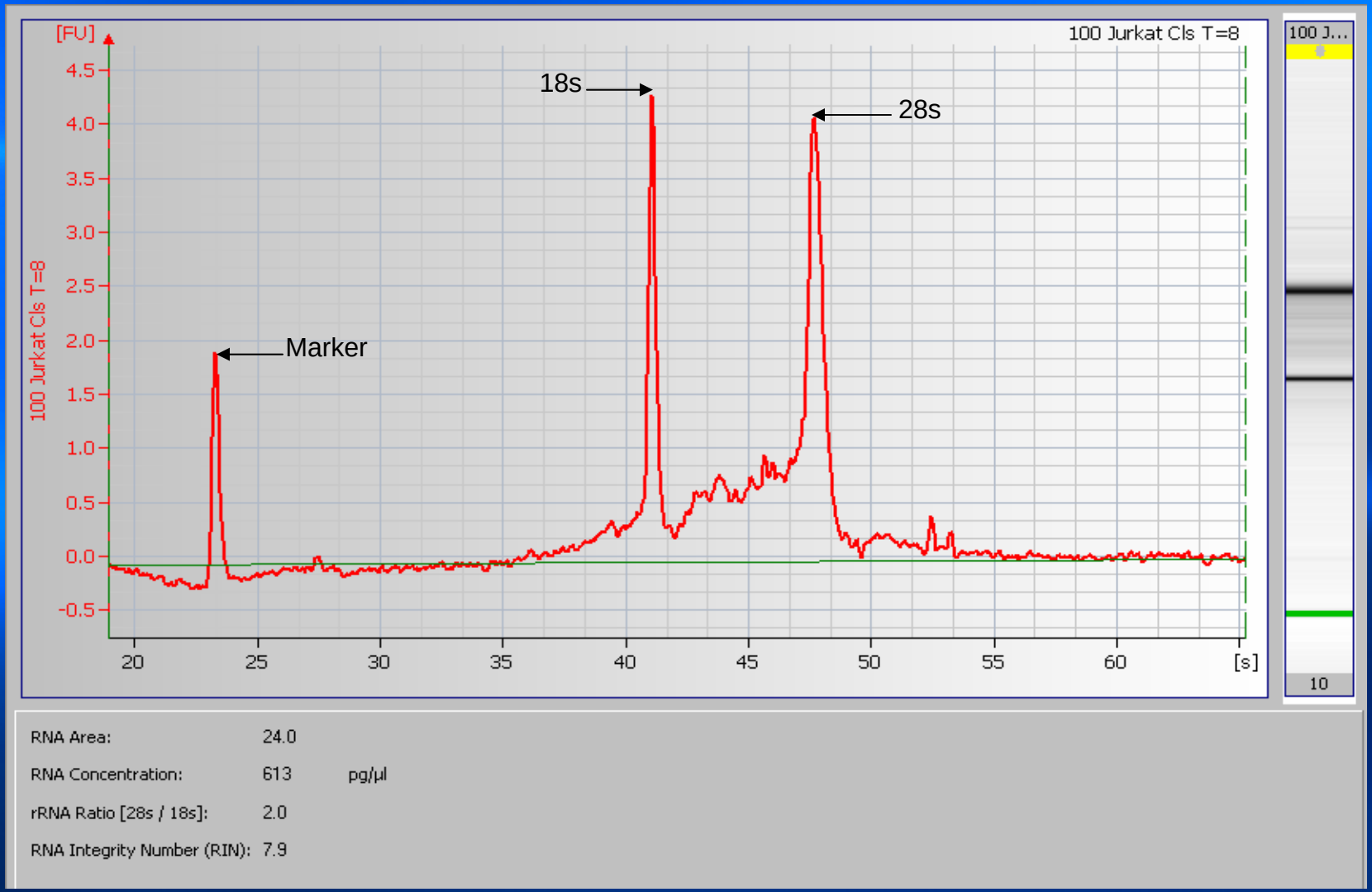
Agilent 2100 Bioanalyzer Capillary Electrophoresis of RNA in SideStep Lysate of HeLa Cells Stored 6 Months at -20°C



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

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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/µl
rRNA Ratio [28s / 18s]:	2.0	
RNA Integrity Number (RIN):	7.9	

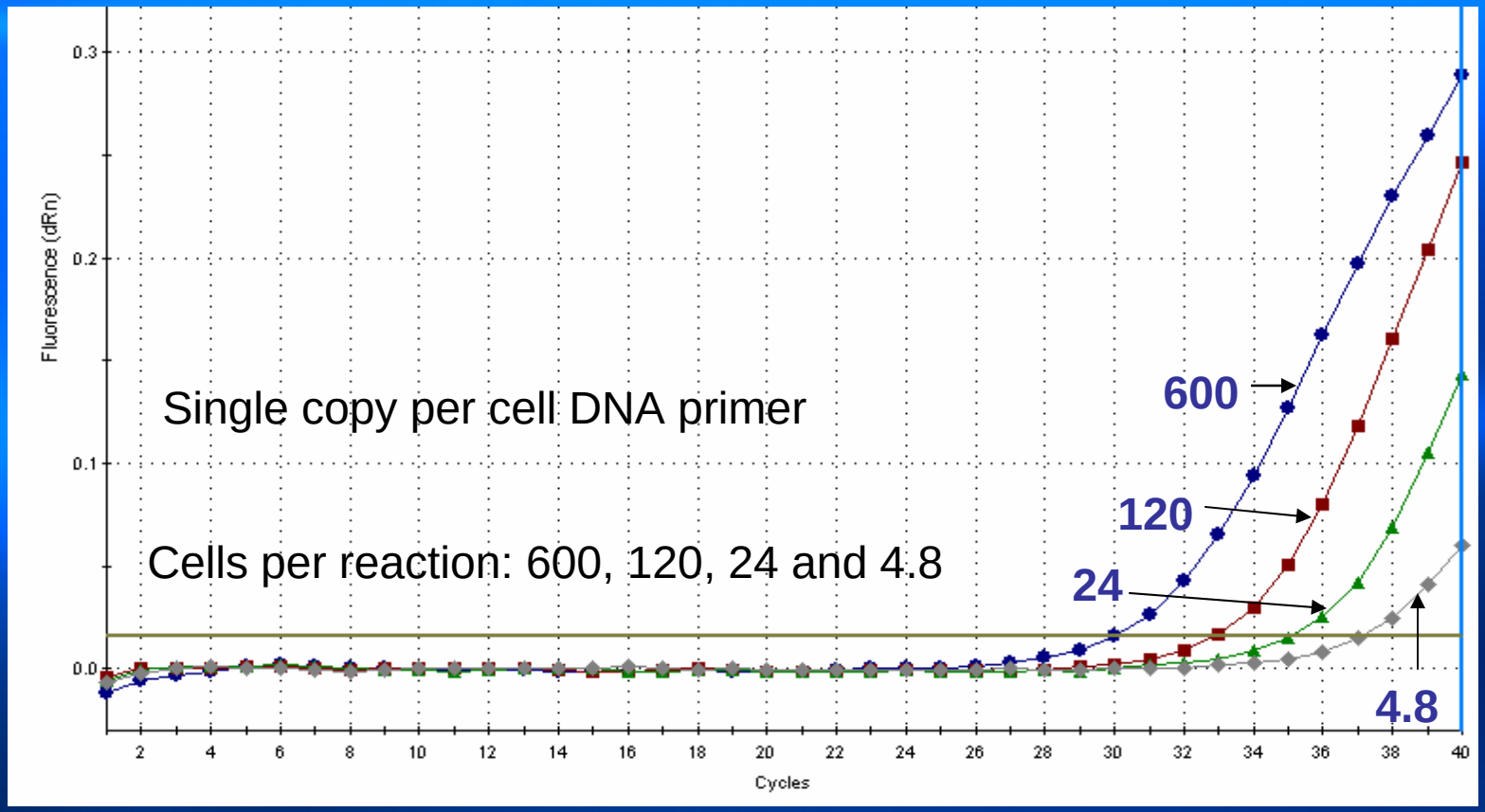
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QPCR with SYBR Green

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

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SideStep Lysate in Brilliant SYBR Green QPCR



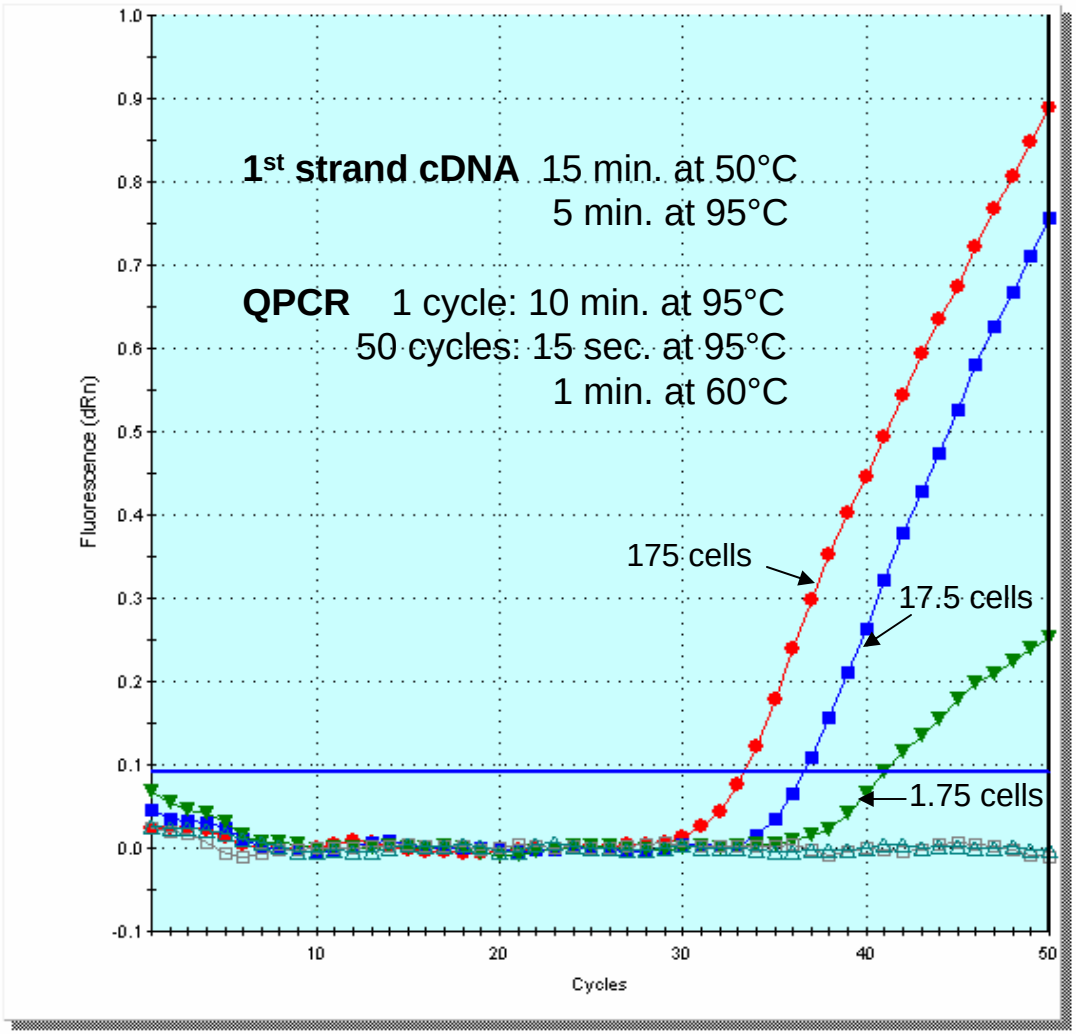
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SideStep in 1st Strand cDNA Synthesis

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

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SideStep Low Abundance Gene Expression



BAX Primer Probe

700 HeLa cells in 1st strand, 5ul in QPCR
700 HeLa cells in 1st strand, 0.5ul in QPCR
700 HeLa cells in 1st strand, 0.05ul in QPCR
NoRT
No Template Control

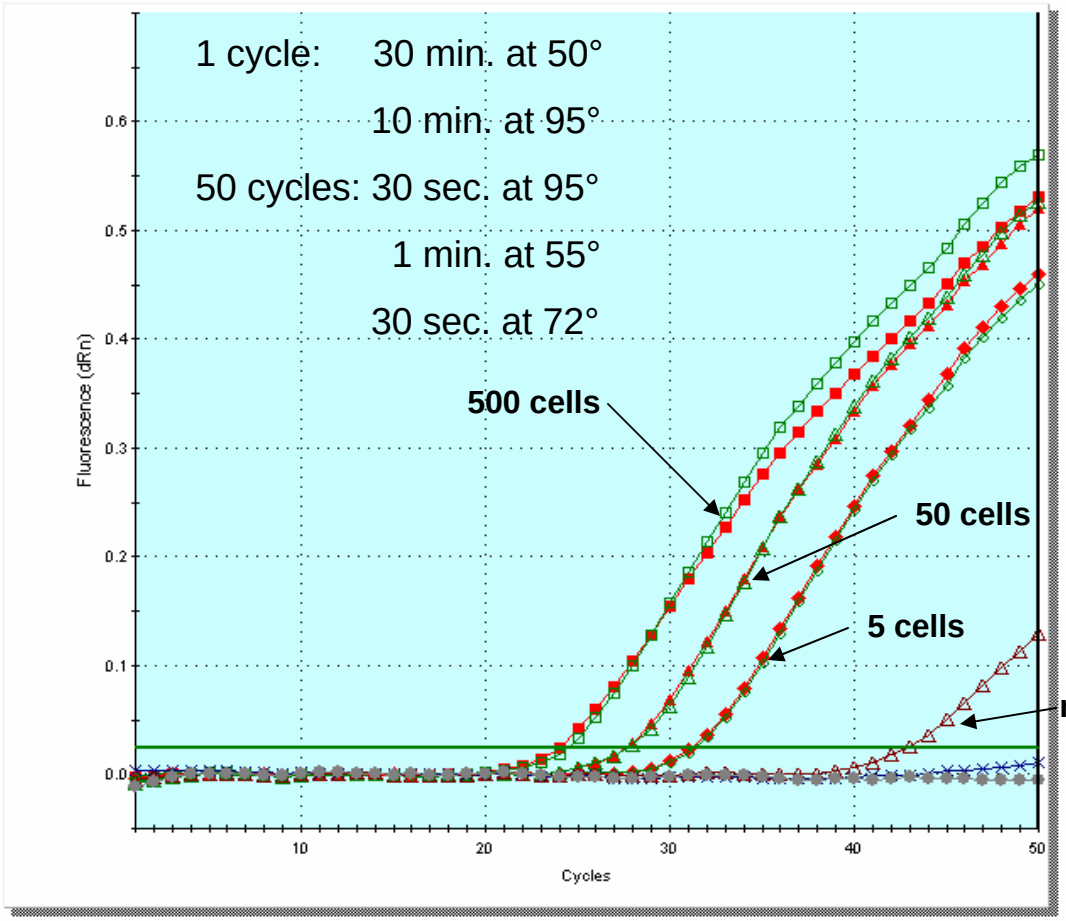
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Absolutely mRNA isolation from SideStep lysate

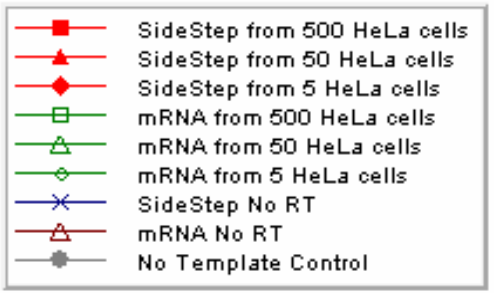
- Simple addition to Absolutely mRNA isolation protocol
- Allows for Magnetic mRNA isolation from cells
- Signal from SideStep lysate equal to isolated mRNA

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SideStep vs. Isolated mRNA

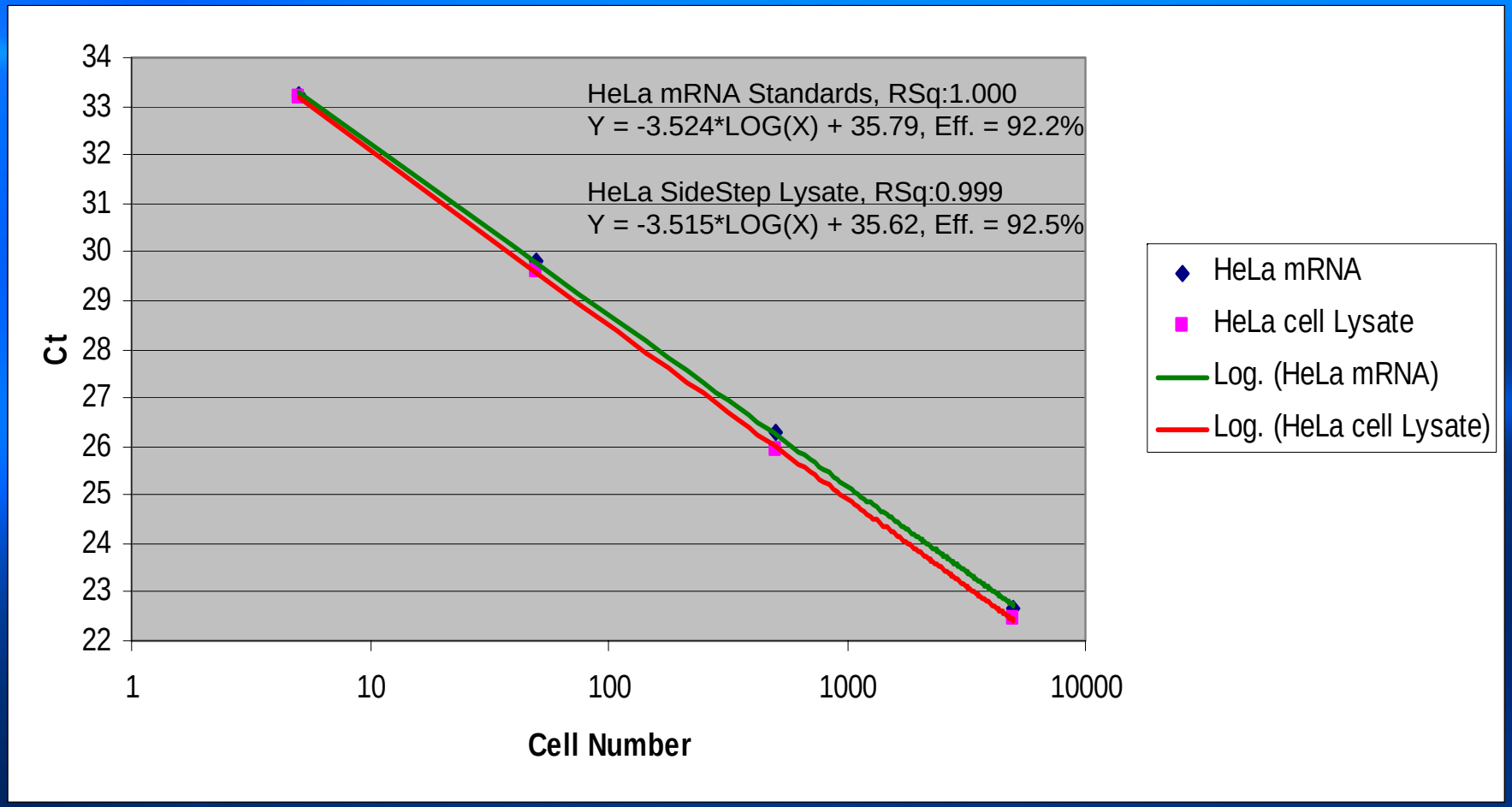


GAPDH Primer Probe



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



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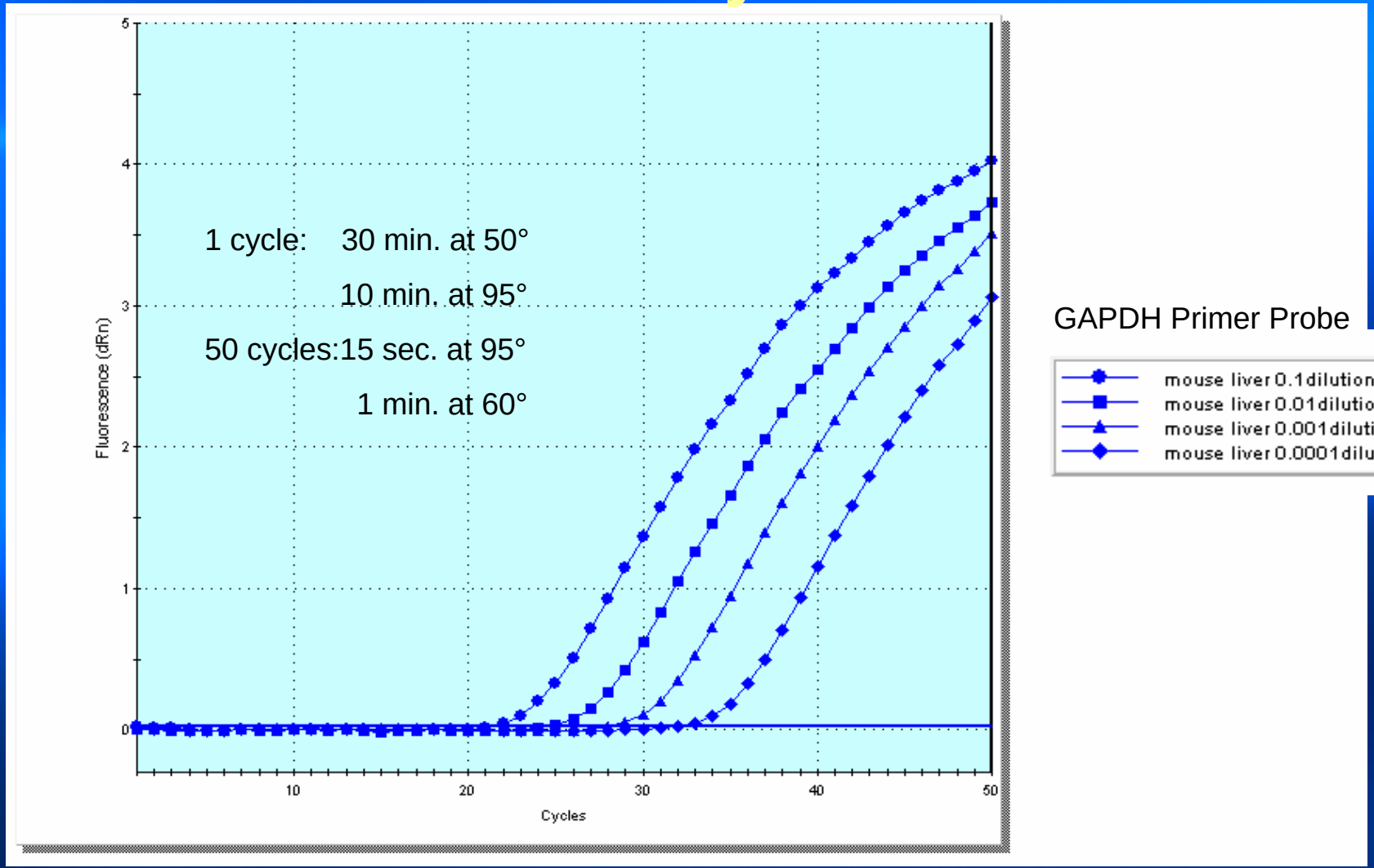
SideStep Tissue lysate Preparation

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



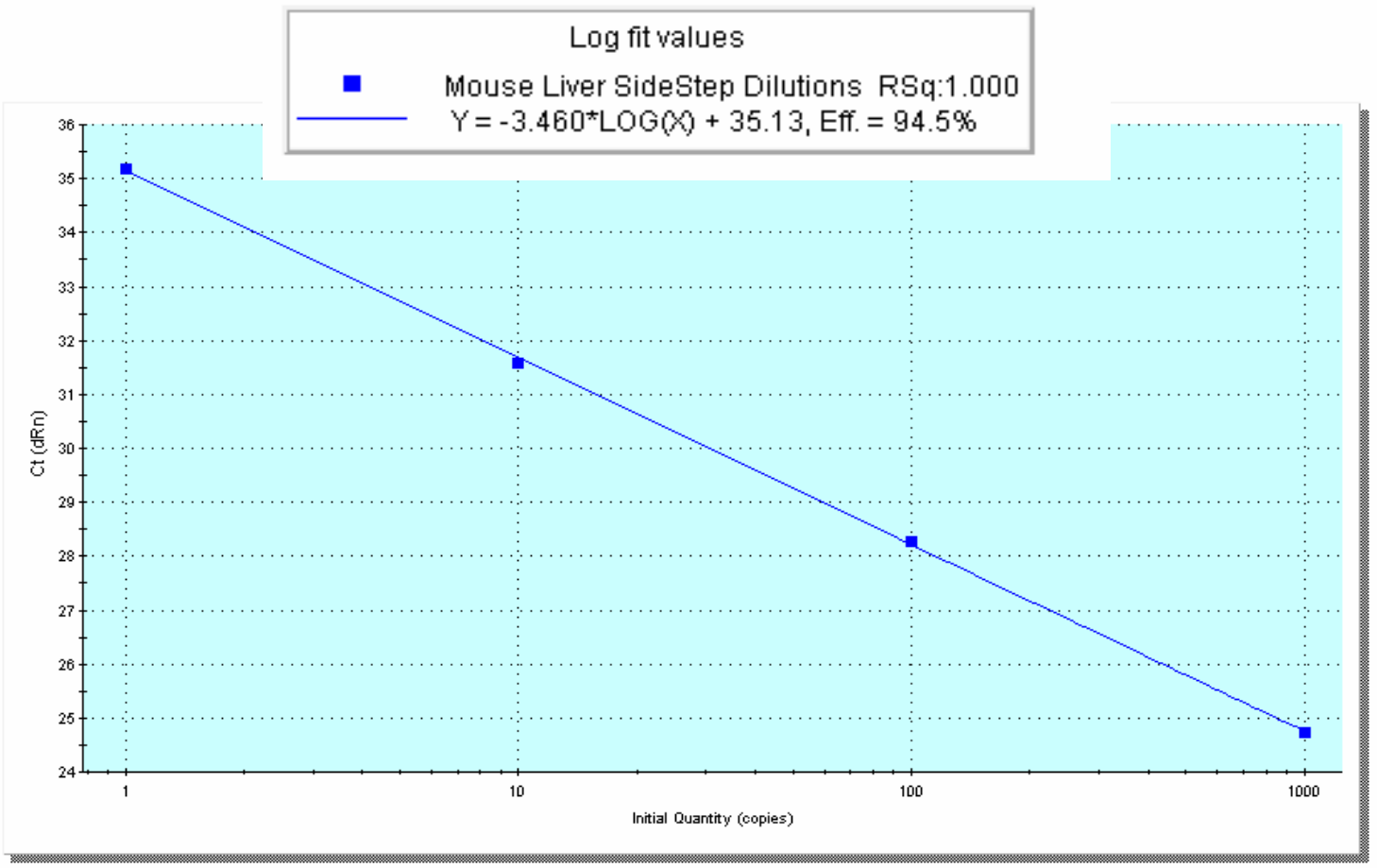
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GAPDH Expression in SideStep Mouse Liver Lysate



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Standard Curve for Mouse Liver in SideStep



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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

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