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
Regardless of initial lysate concentration, equal cell numbers give equal Ct’s.
Signal from SideStep is Equal to Purified RNA

1 cycle: 30 min. at 50°
10 min. at 95°
50 cycles: 30 sec. at 95°
1 min. at 55°
30 sec. at 72°

GAPDH Primer Probe
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

1 cycle: 30 min. at 50°
10 min. at 95°
50 cycles: 30 sec. at 95°
1 min. at 55°
30 sec. at 72°

GAPDH Primer Probe
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
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
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

Single copy per cell DNA primer

Cells per reaction: 600, 120, 24 and 4.8
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

1st strand cDNA: 15 min. at 50°C, 5 min. at 95°C

QPCR: 1 cycle: 10 min. at 95°C, 50 cycles: 15 sec. at 95°C, 1 min. at 60°C

BAX Primer Probe

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

Fluorescence (dF/dN) vs. Cycles

175 cells, 17.5 cells, 1.75 cells
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

1 cycle: 30 min. at 50°
10 min. at 95°
50 cycles: 30 sec. at 95°
1 min. at 55°
30 sec. at 72°

500 cells
50 cells
5 cells
mRNA No RT

GAPDH Primer Probe

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

HeLa mRNA Standards, RSq: 1.000
Y = -3.524*LOG(X) + 35.79, Eff. = 92.2%

HeLa SideStep Lysate, RSq: 0.999
Y = -3.515*LOG(X) + 35.62, Eff. = 92.5%
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

1 cycle: 30 min. at 50°
10 min. at 95°
50 cycles: 15 sec. at 95°
1 min. at 60°

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

Log fit values

Mouse Liver SideStep Dilutions  R^2 = 1.000
Y = -3.460 \times \log(x) + 35.13, Eff. = 94.5%
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