SideStep Lysis and Stabilization Buffer

INSTRUCTION MANUAL

Catalog #400900
Revision B.0

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400900-12

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SideStep Lysis and Stabilization Buffer

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SideStep Lysis and Stabilization Buffer

MATERIALS PROVIDED

Catalog #400900

Material provided	Quantity
SideStep Lysis & Stabilization Buffer	10 ml

STORAGE CONDITIONS

SideStep Lysis and Stabilization Buffer: Store at –20°C upon receipt. After thawing, store at 4°C.

ADDITIONAL MATERIALS REQUIRED

PBS, cold (see *Preparation of Media and Reagents*)
RNase- and DNase-free H_2O
Microcentrifuge tubes

Revision B.0

INTRODUCTION

SideStep Lysis and Stabilization Buffer allows you to go from mammalian cells to QPCR or QRT-PCR experiments without any nucleic acid purification steps.

QRT-PCR is an important technique for studying mRNA levels in biological samples. Conventional QRT-PCR experiments include RNA isolation steps in order to protect the mRNA of interest from degradation by cellular RNases and to remove inhibitors of reverse transcriptase from the sample. The process of RNA isolation is tedious and time-consuming, particularly when processing large numbers of samples. In addition, RNA is subject to loss during isolation procedures, which is especially problematic when working with small samples and low-abundance RNAs.

Overview of SideStep Technology

Using SideStep technology*, you can skip the nucleic acid purification steps in your QRT-PCR experiments, making analysis of a large number of samples much faster and simpler. The SideStep technology achieves cell lysis and nucleic acid stabilization in the same buffer, eliminating the need for RNA purification. The simple protocol takes approximately 10 minutes to perform and includes a single PBS wash followed by cell lysis in the SideStep lysis buffer. This buffer inactivates cellular nucleases and other enzymes, and the nucleic acids released into the buffer are stabilized and suitable for QPCR or QRT-PCR analysis for at least 20 months when stored at –80°C.

The long-range stability of SideStep lysates offers the potential to perform multiple experiments using the same sample and to archive samples of interest for further analysis or RNA isolation. RNA may be isolated from SideStep lysates using most standard RNA purification methods. The SideStep mRNA enrichment kit (Catalog #400902) combines the SideStep buffer with an oligo-dT magnetic bead-based mRNA purification kit, offering a particularly convenient RNA purification option.

*Patent pending.

Applications

SideStep technology may facilitate any application where nucleic acids are analyzed from cultured mammalian cells. The technology is particularly well suited for applications involving the analysis of RNA or DNA levels in a large number of samples. Examples include the following applications:

- siRNA knockdown detection
- miRNA detection
- mRNA profiling in cell differentiation or drug treatment experiments
- screening of compounds for effects on target mRNA levels
- time-course experiments
- screening of samples for the presence/absence of DNA or RNA target

For your convenience, we offer the SideStep buffer in combination with a variety of downstream analysis kits. See the table below for available combinations.

SideStep Kits for Specific Analysis Applications

Application	Product	Catalog #
QRT-PCR	SideStep II QRT-PCR Master Mix, 1-Step	400917
	SideStep II QRT-PCR Master Mix, 2-Step	400918
	SideStep II SYBR® Green QRT-PCR Master Mix, 2-Step	400909
QPCR	SideStep SYBR® Green QPCR Master Mix	400904
mRNA Purification	SideStep mRNA Enrichment Kit	400902
cDNA synthesis directly from cell lysates	SideStep II QPCR cDNA Synthesis Kit	400908

PREPROTOCOL CONSIDERATIONS

Storage of SideStep Lysates and Dilutions

The SideStep system allows long-term storage of cell lysates. The **undiluted** lysates may be stored at 4°C for 1 month, at –20°C for 6 months, or at –80°C for 20 months. When dilution of SideStep lysates is necessary for use in downstream applications, dilute lysates in nuclease-free water and use immediately. Since nucleic acids are no longer stabilized after dilution of the SideStep buffer, do not store the lysate dilutions for future analysis.

QPCR and QRT-PCR Assay Considerations

Primer Design

Design QPCR or QRT-PCR primers to generate amplicons of ≤150 bp.

For QRT-PCR experiments, primers should be designed to prevent amplification of genomic DNA. One approach is to include a primer that spans an exon-exon boundary in the target mRNA. This primer will not bind to genomic DNA sequences, where an intron interrupts the primer binding site. A second approach is to use primers that flank a large intron. Using this approach, a small amplicon (≤150 bp) is amplified from the intronless cDNA, but amplification of the large intron-containing genomic DNA amplicon does not occur under the cycling conditions used.

When possible, design primers to avoid regions of secondary structure in the mRNA.

Probe and Protocol Selection

SideStep lysates may be analyzed using linear probes (e.g. TaqMan® probes) in either one-step or two-step QRT-PCR protocols. For SYBR® Green detection in QRT-PCR, use a two-step protocol. QPCR may be performed using either probe- or SYBR Green-based detection.

Optimizing QPCR and QRT-PCR Assays

Prior to performing a large-scale experiment or screen using QPCR or QRT-PCR analysis of lysates, the assay should be optimized for the specific target of interest and the specific primer/probe system using purified RNA. Important optimization parameters include primer and probe concentrations and PCR cycling conditions. Stratagene QPCR Reference Total RNA, purchased separately, provides an ideal source of RNA for assay optimization (human reference RNA, Catalog #750500 and mouse reference RNA, Catalog #750600).

PROTOCOL

Note *The SideStep lysis and stabilization buffer may be used to prepare lysates from a variety of mammalian cell lines. Commonly used cell culture and harvesting methods are compatible with the SideStep buffer protocols, and those methods routinely used by your laboratory for the specific cell line should be employed.*

Cell Density Considerations

Lysates may be prepared with cell densities of up to 10^4 cell equivalents/µl of lysis buffer. When sufficient cultured cells are available, We recommend preparing the lysate at the maximum cell density (10^4 cells/µl) for maximum flexibility in downstream applications.

Cells are washed once in cold PBS in the protocol below. The density of the PBS suspension in step 4 will equal the final cell density of the lysate.

Prior to performing a large-scale experiment or screen using QPCR or QRT-PCR analysis of lysates, perform a pilot standard curve to determine the cell number range that gives linear amplification of the specific target under your specific reaction conditions.

Preparation of Lysates from Cultured Cells

1. Harvest the cultured cells, using a method appropriate to the properties of the cell line and the growth vessel.

   Note *If trypsin is used for cell harvesting, it must be inactivated before proceeding.*

2. Count the cells in an aliquot of the cell suspension.

3. Pellet the cells at 500–1,000 × *g* at 4°C for 5 minutes. Aspirate the supernatant.

4. Resuspend the pelleted cells in at least 100 µl of cold PBS to a final concentration of $\leq 10^4$ cells/µl.

   Note *The density of the PBS cell suspension will equal the final cell density in the lysate. We recommend using the maximum cell density of 10^4 cells/µl, for maximum flexibility in downstream applications.*

5. Place 100 µl of the cell suspension ($\leq 10^6$ cells) in a 1.5-ml microfuge tube.

6. Pellet the cells at 500–1,000 × *g* at 4°C for 5 minutes. Aspirate the supernatant. Remove as much of the PBS as possible without disturbing the pellet.

7. Add 100 µl of SideStep lysis and stabilization buffer to the cell pellet.

8. Vortex for 1 minute to lyse the cells.

9. Process the lysate using the appropriate application or store the lysate according to the following considerations. Nucleic acids in the lysate are stable at room temperature for 4 hours, at 4°C for 1 month, at –20°C for 6 months, and at –80°C for at least 20 months. For QRT-PCR applications, see *QRT-PCR Guidelines*, below, for lysate dilution and processing guidelines.

QRT-PCR Guidelines

One-Step QRT-PCR

Note ***One-step QRT-PCR analysis of SideStep lysates should be performed using probe-based detection methods only.*** *For SYBR Green dye-based detection, perform two-step QRT-PCR experiments.*

The amount of lysate added to the QRT-PCR reaction depends on the experimental design (target abundance and the desired cell equivalents per reaction). Keep the following considerations in mind when planning one-step QRT-PCR analysis using SideStep lysates.

High concentrations of either cellular materials or lysis buffer may inhibit the QPCR reaction. The number of cell equivalents added to a 25-µl QRT-PCR reaction should not exceed 100 and the total volume of **undiluted** lysate should not exceed 1 µl. However, for some cell lines, up to 200 cell equivalents may be used. Run a standard curve, analyzing serial dilutions of cell equivalents, to determine the maximum number of cells for your cell line Typically, lysates prepared at 10^4 cells/µl are serially diluted in water prior to addition to one-step QRT-PCR reactions The chart below illustrates options for addition of different cell number equivalents.

Desired Cell Equivalents	Cell Density of Undiluted Lysate		
	10^4 cells/µl	10^3 cells/µl	10^2 cells/µl
200	1 µl of 1:50 dilution	1 µl of 1:5 dilution	—
100	1 µl of 1:100 dilution	1 µl of 1:10 dilution	1 µl (undiluted lysate)
50	1 µl of 1:200 dilution	1 µl of 1:20 dilution	1 µl of 1:2 dilution
25	1 µl of 1:400 dilution	1 µl of 1:40 dilution	1 µl of 1:4 dilution
12.5	1 µl of 1:800 dilution	1 µl of 1:80 dilution	1 µl of 1:8 dilution
6.25	1 µl of 1:1,600 dilution	1 µl of 1:160 dilution	1 µl of 1:16 dilution
3.125	1 µl of 1:3,200 dilution	1 µl of 1:320 dilution	1 µl of 1:32 dilution

Note *Storage of diluted cell lysates is not recommended.*

The lower limit for SideStep lysate addition to QRT-PCR reactions is determined by the abundance of the target and the sensitivity of the assay system used.

Two-Step QRT-PCR

DNase Treatment

For two-step QRT-PCR using a nonspecific double-stranded DNA binding dye, such as SYBR Green, for detection, treatment of SideStep lysates with DNase prior to cDNA synthesis is strongly recommended. For two-step protocols with probe-based detection, DNase treatment is not necessary if the primers are designed to avoid amplification of genomic DNA. The SideStep lysis buffer is offered in combination with a RNase-free DNase I enzyme in the SideStep II Cell Lysis Analysis Kit (catalog #400916) if removal of genomic DNA from your lysate samples is required.

Amount of Lysate to Use as Template

For first-strand cDNA synthesis, the total number of cell equivalents added to a 20-µl reaction should not exceed 100 and the volume of **undiluted** lysate added to the reaction should not exceed 1 µl. The addition of more than 100 cell equivalents or more than 1 µl of 1× SideStep buffer may inhibit reverse transcription. Additionally, a 25-µl QPCR reaction should contain no more than 2 µl of the cDNA synthesis reaction, or 10 cell equivalents. (This is a general guideline; for some cell lines, up to 200 cell equivalents may be added to the cDNA synthesis reaction without inhibiting reverse transcription and, subsequently, the QPCR reaction can then accommodate up to 20 cell equivalents. Analyze serial dilutions of your cDNA samples to determine the maximum number of cells that may be added to the first-strand synthesis reaction.)

Example *If lysates are prepared at 10^4 cell equivalents/µl, the following protocol may be used. Use nuclease-free water to dilute the SideStep lysate 1:100 for a final concentration of 100 cell equivalents/µl, and then immediately add 1 µl of the diluted lysate to a 20-µl cDNA synthesis reaction. In this example, the cDNA synthesis reaction will contain 100 cell equivalents. The 25-µl QPCR reaction, containing 2 µl of the cDNA synthesis reaction, will then contain 10 cell equivalents. When running a standard curve, make 2-fold serial dilutions of the cDNA synthesis reaction such that cell equivalents of 10, 5, 2.5 and 1.25 are analyzed.*

TROUBLESHOOTING

Observations	Suggestions
No or low amounts of PCR products detected after QPCR or QRT-PCR	Optimize the QPCR or QRT-PCR assay. Important optimization parameters include primer design, primer concentration, probe design and probe concentration.
	For best results, design primers that produce amplicons <150 bp in length.
	Increase the number of PCR cycles.
	Determine the optimal primer binding temperature for the PCR cycling program.
	RNA may have been degraded prior to the addition of SideStep lysis buffer. To prevent degradation, ensure that cold PBS is used to wash the cells, and that the cells are kept on ice prior to lysis buffer addition.
	The addition of too many cell-equivalents to the PCR reaction may be inhibitory. Prepare the lysates at $\leq 10^4$ cells/µl, and follow the guidelines in *QRT-PCR Guidelines* for the upper limits of lysate addition. If inhibition of QPCR by the addition of too many cell equivalents is suspected, the cell lysates may be diluted in water prior to addition to the QPCR or QRT-PCR reaction.
	Verify that the QRT-PCR protocol successfully amplifies the target RNA from a positive control sample, such as the Stratagene QPCR Human Reference RNA (catalog #750500).
Unexpected PCR products	Optimize primer design and the primer annealing temperature.
	Alternatively-spliced forms of the transcript may be present. Redesign primers to another part of the mRNA.
PCR products detected in no-RT negative controls	Genomic DNA in the lysate may be amplified in the absence of RT. Redesign primers to span an exon-exon boundary or to flank a large intron to avoid amplification of genomic DNA. If using SYBR Green for detection, treat the lysate with RNase-free DNase I.

PREPARATION OF MEDIA AND REAGENTS

PBS (Phosphate Buffered Saline)

150 mM NaCl
20 mM Na_2HPO_4
adjust to pH 7.4 with HCl

ENDNOTES

SYBR® is a registered trademark of Molecular Probes, Inc.
TaqMan® is a registered trademark of Roche Molecular Systems, Inc.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.